

Report
of
Test Nos. 865-1, 2 & 4
Toxicity of Marine Sediments Using 10-day *Eohaustorius estuarius*, 20-day *Neanthes*
***arenaceodentata* and *Mytilus provincialis* Larval Sediment Bioassays as Part of the**
Port of Seattle – West Waterway Deepening Project

Submitted to

Anchor QEA
720 Olive Way, Suite 1900
Seattle, Washington 98101

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

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EXECUTIVE SUMMARY OF SEDIMENT BIOASSAYS

Three marine sediment bioassays, a 10-day *Eohaustorius estuarius* survival test, a 20-day *Neanthes arenaceodentata* survival and growth test and a 48-hour *Mytilus provincialis* larval test, were conducted for Anchor QEA as part of the Port of Seattle – West Waterway Deepening project. Four test sediments were compared to the reference sediment or negative control sediment to assess sediment toxicity and to interpret organism response under the one-hit/two-hit rules described in “Dredged Material Evaluation and Disposal Procedures User Manual,” December 2014. The test and reference sediments tested are listed in Table 1.

TEST, REFERENCE AND CONTROL SEDIMENT INFORMATION

The test and reference sediments were provided to Northwestern Aquatic Sciences by Anchor QEA. The negative control sediment (NAS Sample #5250G) was collected from the *Eohaustorius estuarius* amphipod collection site in lower Yaquina Bay, Oregon, on 3-25-15. All sediments were stored at 4°C in the dark until used. Sample identification and collection information is as follows:

Table 1. Sample identification and collection dates.				
Sample description	Anchor QEA Sample Identification	NAS Sample Identification	Collection Date	Receipt Date
Negative Control	Control	5250G	3-25-15	3-25-15
Test sediment	WWD02-0-4-150205	5246G	2-5-15	3-23-15
Test sediment	WWD02-4-8-150205	5245G	2-5-15	3-23-15
Test sediment	WWD05-0-4-150206	5231G	2-6-15	3-23-15
Test sediment	WWD12-0-4-150206	5247G	2-6-15	3-23-15
Reference sediment	WWD-REF-150319	5230G	3-19-15	3-20-15

PRE-PURGE TEST

DMMP agencies recommend determining the need for purging or reference toxicant testing prior to actual bioassay testing. Pre-purge testing was conducted on the test and reference sediments following methods in the 2013 DMMP Clarification Paper, “Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing.” This DMMP Clarification Paper was the most recent available guidance at the time of testing. The method involved setting up one beaker for *Eohaustorius/Neanthes* pre-purge testing and one beaker for mussel larval pre-purge testing, in the manner they would be set up for actual bioassay testing, but without test organisms. After the standard settling/equilibration time equivalent to test initiation (the next day for *Eohaustorius/Neanthes* beaker and 4 hours after mixing for the larval beaker), the total ammonia; total sulfides; pH; salinity and temperature were measured in the overlying water. Using these measurements the unionized ammonia and hydrogen sulfide values were calculated. No sediment unionized ammonia or hydrogen sulfide values exceeded the DMMP-established triggers (Section A); therefore, no purging or ammonia reference toxicant testing was required.

BIOASSAY INTERPRETATION CRITERIA

Biological test interpretation as presented in the “Dredged Material Evaluation and Disposal Procedures User Manual” relies upon two levels of observed response in the test organism. These are known as “one-hit” and “two-hit” criteria failures. The bioassay-specific guidelines for each of these response categories are listed below. In general, a one-hit failure is a response that exceeds the bioassay-specific guidelines relative to the negative control and reference sediments and is statistically different from the reference sediment in any one biological test. A two-hit failure exhibits a lower intensity response that must be present in two or more biological tests. In the event of one hit under the single-hit rule or two hits under the two-hit rule, the sediment is judged to be unsuitable for unconfined open-water disposal.

One-Hit Criteria

When any one of the biological tests shows a test sediment response that exceeds the bioassay-specific response guidelines (presented below), and that response is statistically different ($\alpha=0.05$) from the reference, the test sediment is judged to be a one-hit failure and unsuitable for aquatic disposal.

In accordance with the “Dredged Material Evaluation and Disposal Procedures User Manual,” the bioassay-specific response guidelines for evaluating the one hit criteria are as follows:

Amphipod Bioassay. Mean test mortality that is greater than 20 percent over the mean control response, greater than 10 percent (dispersive) or 30 percent (nondispersive) over the mean reference sediment response, and statistically different from the reference ($\alpha = 0.05$).

Juvenile *Neanthes* Bioassay. Mean individual ash-free dry weight growth rate in the test sediment is less than 80 percent of the mean negative control growth rate, and less than 70 percent (dispersive) or 50 percent (nondispersive) of the reference growth rate, and statistically different from reference ($\alpha = 0.05$).

Sediment larval Bioassay. The test and reference sediment responses are normalized to the negative seawater control response (NCMA). A single-hit failure is indicated when the NCMA in the test sediment is greater than 20 percent, and is 15 percent (dispersive) or 30 percent (nondispersive) over the reference, and statistically different from reference ($\alpha = 0.1$).

Two-Hit Criteria

For marine sediment testing, when any two biological tests exhibit test sediment responses which are less than the criteria for one-hit failures, but are statistically different from reference ($\alpha = 0.05$) (and less than 70 percent of the reference growth rate for the *Neanthes* bioassay for nondispersive sites), the test sediment is judged to be a two-hit failure and unsuitable for open-water disposal.

RESULTS OF INDIVIDUAL BIOASSAYS

Amphipod, *Eohaustorius*, 10-Day Survival Test (865-1)

All water quality observations were within the protocol specified ranges except for several salinity measurements that slightly exceeded the specified range (Table 1, Section B). The test met all other applicable acceptability criteria including positive control performance (Section B).

The control and reference sediments exhibited mean mortalities of 1.0% and 5%, respectively. Two test sediments, WWD02-0-4-150205 and WWD05-0-4-150206 exhibited a mortality that was statistically greater than the reference and exceeded the dispersive disposal guideline for greater than 20% over that of the control and greater than 10% over the reference, and, therefore, met the criteria for failure under the dispersive one-hit rule but did not meet the criteria for the nondispersive one-hit rule for open water disposal (Table 2).

Neanthes 20-Day Survival and Growth Test (865-2)

All water quality observations were within the protocol specified ranges (Table 1, Section C). The average initial weight of worms was 0.48mg, slightly below the recommended range (0.5 to 1.0 mg). The test met all other applicable acceptability criteria including positive control performance (Section C).

The individual ash-free growth rates in the control and the reference sediments *Neanthes* test were 0.58 and 0.65 mg/day, respectively. None of the growth rates for any of the test sediments failed the single-hit criteria for dispersive or non-dispersive disposal (Table 3).

Mussel, *Mytilus galloprovincialis*, Larval Test (865-4)

All water quality observations were within the protocol specified ranges (Table 1, Section D). The test met all other applicable acceptability criteria including positive control performance (Section D).

The test met the control acceptance criterion of $\geq 70\%$ normal in the seawater control; the control mean percent normality was 75.8%. The percent normal of the reference sediment, WWD-REF-150319 was 89.8% of the seawater control. The number normal was used for data analysis and interpretation rather than the NCMA due to the NCMA resulting in negative numbers in some replicates. None of the test sediments was statistically significantly lower than the reference sediment WWD-REF-150319 for number of normal larvae and therefore none failed the single-hit criteria for dispersive or non-dispersive disposal (Table 4).

DATA INTERPRETATION BASED ON "DREDGED MATERIAL EVALUATION AND DISPOSAL PROCEDURES USER MANUAL" CRITERIA

Tables 2-4 present analyses of toxicity test data from Tests No. 865-1, -2, and -4, respectively, according to the one-hit rule. Table 5 summarizes the one- and two-hit rule results. Data interpretation was conducted as described in "Dredged Material Evaluation and Disposal Procedures User Manual," December 2014.

One-hit failures for the dispersive case were observed for two test sediments, WWD02-0-4-150205 and WWD05-0-4-150206 in the *Eohaustorius* test only. None of the test sediments met the criteria for failure under the one-hit nondispersive rule or under the two-hit rule for either dispersive or nondispersive disposal (Table 5).

Table 2. Single-hit criteria interpretation of *Eohaustorius* test data.

Sample description	Percent mortality	Significantly different from the reference at $\alpha = 0.05$?	Percent difference over negative control	Percent difference over the reference	Failure under 1-hit dispersive rule?	Failure under 1-hit nondispersive rule?
Control (NAS# 5250G)	1.0 \pm 2.2	---	---	---	---	---
WWD02-0-4-150205 (NAS# 5246G)	27.0 \pm 7.6	Yes	26.0	22.0	Yes	No
WWD02-4-8-150205 (NAS# 5245G)	7.0 \pm 2.7	No	6.0	2.0	No	No
WWD05-0-4-150206 (NAS# 5231G)	25.0 \pm 11.7	Yes	24.0	20.0	Yes	No
WWD12-0-4-150206 ¹ (NAS# 5247G)	1.0 \pm 2.2	No	0.0	-4.0	No	No
WWD-REF-150319 (NAS# 5230G)	5.0 \pm 3.5	---	---	---	---	---
If the mean test sediment mortality >20% absolute over the mean negative control response, and >10% (dispersive) or >30% (nondispersive) absolute over the mean reference sediment response, and statistically different ($\alpha = 0.05$) from the reference sediment, it fails under the single-hit rule.						
¹ Compared to the control sediment.						

Table 3. Single-hit criteria interpretation of *Neanthes* juvenile infaunal growth test data.

Sample description	Individual ash-free growth rate (mg/day)	Significantly different from the reference at $\alpha = 0.05$?	Significantly different from the control at $\alpha = 0.05$?	Percent of reference value	Percent of negative control	Failure under 1-hit dispersive rule? (T/R <70%)	Failure under 1-hit nondispersive rule? (T/R <50%)
Control (NAS# 5250G)	0.44 ± 0.10	---	---	---	---	---	---
WWD02-0-4-150205 (NAS# 5246G)	0.59 ± 0.06	No	No	120	134	No	No
WWD02-4-8-150205 (NAS# 5245G)	0.57 ± 0.22	No	No	116	130	No	No
WWD05-0-4-150206 (NAS# 5231G)	0.55 ± 0.06	No	No	112	125	No	No
WWD12-0-4-150206 (NAS# 5247G) ¹	0.54 ± 0.07	No	No	110	123	No	No
WWD-REF-150319 (NAS# 5230G)	0.49 ± 0.07	---	---	---	---	---	---
If the mean individual growth rate of a test sediment is <80% of the mean negative control growth rate, and <70% (dispersive) or <50% (nondispersive) of the mean reference sediment growth rate, and statistically different ($\alpha = 0.05$) from the reference sediment, it fails under the single-hit rule.							
¹ Compared to the control sediment.							

Table 4. Single-hit criteria interpretation of *Mytilus* larval sediment bioassay data. The number normal was used for data interpretation rather than the NCMA due to the NCMA resulting in negative numbers in some replicates.

Sample description	Number normal (mean ± SD)	Significantly different from the reference at $\alpha = 0.10$?	Difference from the reference (proportion)	Failure under 1-hit dispersive rule? (Greater than 0.15)	Failure under 1-hit nondispersive rule? (Greater than 0.30)
Seawater control	195 ± 13	---	---	---	---
WWD02-0-4-150205 (NAS# 5246G)	161 ± 11	No	0.07	No	No
WWD02-4-8-150205 (NAS# 5245G)	166 ± 12	No	0.05	No	No
WWD05-0-4-150206 (NAS# 5231G)	151 ± 30	No	0.12	No	No
WWD12-0-4-150206 (NAS# 5247G)	196 ± 12	No	-0.11	No	No
WWD-REF-150319 (NAS# 5230G)	175 ± 45	---	---	---	---
If the mean NCMA for a test sediment is >20% (<80% number normal compared to control), and is 15% (dispersive) or 30% (nondispersive) absolute over the mean reference sediment NCMA (absolute below the mean reference number normal), and statistically different from reference ($\alpha = 0.10$), it fails under the single-hit rule.					

Table 5. Summary of one- and two-hit rule failures. D=dispersive, ND=nondispersive.

	One-Hit Rule Failures* (D/ND)			Two-Hit Rule Failure** (D/ND)
	Test No. 865-1 <i>Eohaustorius</i>	Test No. 865-2 <i>Neanthes</i>	Test No. 865-4 <i>Mytilus</i>	
WWD02-0-4-150205 (NAS# 5246G)	Yes/No	No/No	No/No	No/No
WWD02-4-8-150205 (NAS# 5245G)	No/No	No/No	No/No	No/No
WWD05-0-4-150206 (NAS# 5231G)	Yes/No	No/No	No/No	No/No
WWD12-0-4-150206 (NAS# 5247G) ²	No/No	No/No	No/No	No/No
<p>*When any one of the biological tests shows a test sediment response that exceeds the bioassay-specific response guidelines, and that response is statistically different ($\alpha=0.05$) from the reference, the test sediment is judged to be a one-hit failure and unsuitable for aquatic disposal.</p> <p>**When any two tests show sediment responses which are less than the bioassay-specific guidelines for a one-hit failure, but are statistically significant compared to the reference sediment (and <70% of the mean reference sediment growth rate for the <i>Neanthes</i> bioassay for nondispersive sites), the sediment fails under the two-hit rule and is judged to be unsuitable for unconfirmed open-water disposal.</p>				

REFERENCES

Dredged Material Evaluation and Disposal Procedures USER MANUAL, December 2014. Dredged Material Management Program: Corps of Engineers, Seattle District; Environmental Protection Agency, Region 10; Washington State Department of Natural Resources; and Washington State Department of Ecology.

Inouye, L.; Hoffman, E.; and Fox, D. (2013). Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing. DMMP Clarification Paper.

STUDY APPROVAL

Linda K. Newirth 6/2/15
Assistant Laboratory Director Date

Shahla Lissavir 6/3/15
Project Manager Date

SECTION A

Pre-purge Test

Ammonia in Water or Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD655	Temp (°C)	pH	Salinity (ppt)	Total NH3-N (mg/L)	Unionized NH3 (mg/L)
Blank							
1.0 mg/L NH3-N Std.		0.151				1.00	
3.0 mg/L NH3-N Std.		0.400				3.00	
6.0 mg/L NH3-N Std.		0.810				6.00	
10.0 mg/L NH3-N Std.		1.400				10.00	
3.0 mg/L spike		0.422				3.05	
3.0 mg/L spike dupl.		0.423				3.05	
5.0 mg/L 2nd source		0.700				5.05	
1. 0.5 mL Smpl							
2. 5230G	1	0.014	16.5	7.9	27.0	0.10	0.002 **
3. 5231G	1	0.007	16.5	8.0	27.5	ND	ND **
4. 5245G	1	0.020	16.5	8.0	27.5	0.14	0.004 **
5. 5246G	1	0.009	16.5	8.0	27.5	ND	ND **
6. 5247G	1	0.010	16.5	8.0	27.5	0.07	0.002 **
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$y = 0.1384x$
 $R^2 = 0.9988$

Standard Curve

Total ammonia
Reporting limit (mg/L) = 0.0722

Recovery (%) = 101.7
Precision (RPD) = -0.24
2nd source (%) = 101.1

Sample volume (ml): 0.50
Dilution factor 1

Sample Set Description:
Test No.:
Test Day:
Species:

Sample Type (check)
Proj. No. 865
Bulk Sediment Porewaters
Test Beaker Porewaters
X Overlying Water
Effluent

Pre-purge analysis of overlying ammonia
In larval beakers.

Equals Day 0 overlying water tested after
4 hour sediment settling period w/o aeration.

**** Note: NH3 detection limit is 0.0017 mg/L as N**

Analyst: RSC/JB
Date analysed: 3/25/2015

Ammonia in Water or Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result		Dilution	Temp	pH	Salinity	Total	Unionized
Sample description	factor	OD655	(°C)		(ppt)	NH3-N (mg/L)	NH3 (mg/L)
Blank	---						
1.0 mg/L NH3-N Std.	---	.151				1.00	
3.0 mg/L NH3-N Std.	---	.400				3.00	
6.0 mg/L NH3-N Std.	---	.810				6.00	
10.0 mg/L NH3-N Std.	---	1.40				10.00	
3.0 mg/L spike	---	.422					
3.0 mg/L spike dupl.	---	.423					
5.0 mg/L 2nd source	---	.700					
1 0.5 mL Smpl							
2 5230G	1	.014					
3 5231G	1	.007					
4 5245G	1	.020					
5 5246G	1	.009					
6 5247G	1	.010					
7							
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Standard Curve

Total ammonia

Reporting limit (mg/L) = 0.0722

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor: 1

Sample Set Description:

Test No.:

Test Day:

Species:

Sample Type (check)

Proj. No. 865

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Effluent

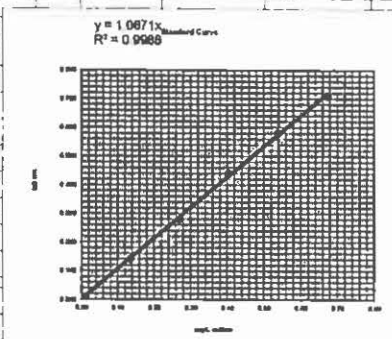
Pre-purge analysis of overlying ammonia
in larval beakers.

Equals Day 0 overlying water tested after
4 hour sediment settling period w/o aeration.

**** Note: NH3 detection limit is 0.0017 mg/L as N**

Analyst: RSC/JB
 Date analysed: 3/25/2015

Dissolved Sulfide in Water or Sediment Pore Water: Computation Worksheet									
Methylene Blue Method (SOP #5550)									
Standardization		1	2	3					
uL PAO titrant employed:		160	153	161					
Working Std. Conc. (mg/L):		0.672							
Result									
Sample description	Dilution factor	OD664	Temp (°C)	pH	Salinity (‰)	Total Sulfide (mg/L*)	H ₂ S (mg/L*)		
Blank									
1.0 mL working sulfide std.		0.140				0.13			
2.0 mL working sulfide std.		0.279				0.27			
3.0 mL working sulfide std.		0.440				0.40			
4.0 mL working sulfide std.		0.580				0.54			
5.0 mL working sulfide std.		0.710				0.67			
3.0 mL spike		0.437				0.41			
3.0 mL spike dupl.		0.440				0.41			
1 5.0 mL Smp									
2 5230G	1	0.002	16.5	7.9	27.0	ND	ND	**	Total Sulfide
3 5231G	1	0.001	16.5	8.0	27.5	ND	ND	**	Reporting limit (mg/L) = 0.0200
4 5245G	1	0.000	16.5	8.0	27.5	ND	ND	**	
5 5246G	1	0.001	16.5	8.0	27.5	ND	ND	**	Recovery (%) = 101.9
6 5247G	1	0.000	16.5	8.0	27.5	ND	ND	**	Precision (RPD) = -0.68
7									
8									
9									
10									Sample volume (ml): 5.00
11									Dilution factor 1
12									
13									Sample Set Description:
14									Test No.:
15									Test Day:
16									Species:
17									
18									Sample Type (check):
19									Proj. No.: 865
20									Bulk sediment porewaters
21									Test beaker porewaters
22									X Overlying water
23									
24									Pre-purge analysis of overlying sulfide in larval beakers.
25									
26									Equals Day 0 overlying water tested after 4 hour sediment settling period w/o aeration.
27									
28									
29									**Note: H ₂ S detection limit is 0.0008 mg/L as S
30									
31									
32									
33									
34									
35									
36									
37									
38									Analyst: RSC/JB
39									Date analysed: 3/25/2015
40									
* mg/L as sulfur									



Dissolved Sulfide in Water or Sediment Pore Water: Computation Worksheet									
Methylene Blue Method (SOP #5550)									
Standardization		1	2	3					
μL PAO titrant employed:		160	153	161					
Working Std. Conc. (mg/L):		3.2							
Result									
Sample description	Dilution factor	OD664	Temp (°C)	pH	Salinity (‰)	Total Soluble Sulfide (mg/L*)	H ₂ S (mg/L*)		
Blank	---	---	---	---	---	---	---		
1.0 mL working sulfide std.	---	.140	---	---	---	0.64	---		
2.0 mL working sulfide std.	---	.279	---	---	---	1.28	---		
3.0 mL working sulfide std.	---	.440	---	---	---	1.92	---		
4.0 mL working sulfide std.	---	.580	---	---	---	2.56	---		
5.0 mL working sulfide std.	---	.710	---	---	---	3.20	---		
3.0 mL spike	---	.437	---	---	---	---	---		
3.0 mL spike dupl.	---	.440	---	---	---	---	---		
1 5.0 mL Smpl	---	---	---	---	---	---	---		
2 5230G	1	.002	---	---	---	---	---		
3 5231G	1	.001	---	---	---	---	---		
4 5245G	1	.000	---	---	---	---	---		
5 5246G	1	.001	---	---	---	---	---		
6 5247G	1	.000	---	---	---	---	---		
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39	---	---	---	---	---	---	---		
40	---	---	---	---	---	---	---		
* mg/L as sulfur									

Standard Curve

ND ** Total Sulfide

ND ** Reporting limit (mg/L): 0.0200

ND ** Recovery (%) = #VALUE!

ND ** Precision (RPD) = #VALUE!

Sample volume (ml): 5.00

Dilution factor: 1

Sample Set Description:

Test No.:

Test Day:

Species:

Sample Type (check):

Proj. No.: 865

Bulk sediment porewaters

Test beaker porewaters

X Overlying water

Pre-purge analysis of overlying sulfide in larval beakers.

Equals Day 0 overlying water tested after 4 hour sediment settling period w/o aeration.

****Note: H₂S detection limit is 0.0008 mg/L as S**

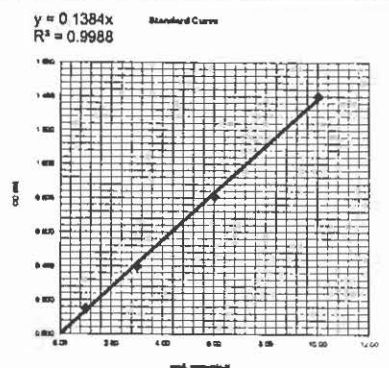
Analyst: RSC/JB *RSC*

Date analysed: 3/25/2015

Ammonia in Water or Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD655	Temp (°C)	pH	Salinity (ppt)	Total NH3-N (mg/L)	Unionized NH3 (mg/L)
Blank	—	—				—	—
1.0 mg/L NH3-N Std.	—	0.151				1.00	
3.0 mg/L NH3-N Std.	—	0.400				3.00	
6.0 mg/L NH3-N Std.	—	0.810				6.00	
10.0 mg/L NH3-N Std.	—	1.400				10.00	
3.0 mg/L spike	—	0.422				3.05	
3.0 mg/L spike dupl.	—	0.423				3.05	
5.0 mg/L 2nd source	—	0.700				5.05	



1	0.5 mL Smpl						
2	5230G	1	0.061	16.5	7.9	27.0	0.44 0.011**
3	5231G	1	0.012	16.4	8.0	27.0	0.09 0.003**
4	5245G	1	0.030	16.4	8.0	27.0	0.22 0.006**
5	5246G	1	0.011	16.3	8.0	27.0	0.08 0.002**
6	5247G	1	0.009	16.4	7.9	27.0	ND ND**

Total ammonia
 Reporting limit (mg/L) = 0.0722

Recovery (%) = 101.7
 Precision (RPD) = -0.24
 2nd source (%) = 101.1

Sample volume (ml): 0.50
 Dilution factor 1

Sample Set Description:

Test No.:
 Test Day:
 Species:

Sample Type (check)

Proj. No. 865
 Bulk Sediment Porewaters
 Test Beaker Porewaters
☒ Overlying Water
 Effluent

Pre-purge analysis of overlying ammonia
In amphipod/worm beakers.

Equals Day 0 overlying water in normal test.

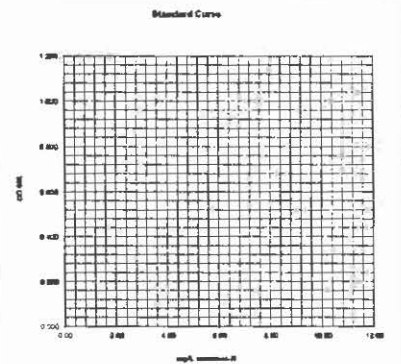
**** Note: NH3 detection limit is 0.0017 mg/L as N**

Analyst: RSC/JB
 Date analysed: 3/25/2015

Ammonia in Water or Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD655	Temp (°C)	pH	Salinity (ppt)	Total NH3-N (mg/L)	Unlonized NH3 (mg/L)
Blank	---	---					
1.0 mg/L NH3-N Std.	---	0.151				1.00	
3.0 mg/L NH3-N Std.	---	0.400				3.00	
6.0 mg/L NH3-N Std.	---	0.810				6.00	
10.0 mg/L NH3-N Std.	---	1.400				10.00	
3.0 mg/L spike	---	0.422					
3.0 mg/L spike dupl.	---	0.423					
5.0 mg/L 2nd source	---	0.700					



1	0.5 mL Smpl						
2	5230G	1	0.061				
3	5231G	1	0.012				
4	5245G	1	0.030				
5	5246G	1	0.011				
6	5247G	1	0.009				

Total ammonia
 Reporting limit (mg/L) = 0.1000

Recovery (%) = #VALUE!
 Precision (RPD) = #VALUE!
 2nd source (%) = #VALUE!

Sample volume (ml): 0.50
 Dilution factor 1

Sample Set Description:

Test No.:
 Test Day:
 Species:

Sample Type (check)

Proj. No. 865
 Bulk Sediment Porewaters
 Test Beaker Porewaters
☒ Overlying Water
 Effluent

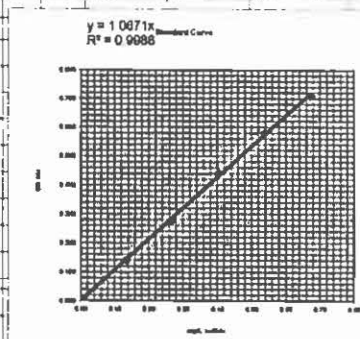
Pre-purge analysis of overlying ammonia
 in amphipod/worm beakers.

Equals Day 0 overlying water in normal test.

Analyst: RSC/JB
 Date analysed: 3/25/2015

83

Dissolved Sulfide in Water or Sediment Pore Water: Computation Worksheet									
Methylene Blue Method (SOP #5550)									
Standardization		1	2	3					
μL PAO titrant employed:		160	153	161					
Working Std. Conc. (mg/L):			0.672						
Result									
Sample description	Dilution factor	OD664	Temp (°C)	pH	Salinity (‰)	Total Soluble Sulfide (mg/L*)	H ₂ S (mg/L*)		
Blank									
1.0 mL working sulfide std.		0.140				0.13			
2.0 mL working sulfide std.		0.279				0.27			
3.0 mL working sulfide std.		0.440				0.40			
4.0 mL working sulfide std.		0.580				0.54			
5.0 mL working sulfide std.		0.710				0.67			
3.0 mL spike		0.437				0.41			
3.0 mL spike dupl.		0.440				0.41			
1 5.0 mL Smpl									
2 5230G	1	0.001	16.5	7.9	27.0	ND	ND	**	Total Sulfide
3 5231G	1	0.001	16.4	8.0	27.0	ND	ND	**	Reporting limit (mg/l) 0.0093
4 5245G	1	0.000	16.4	8.0	27.0	ND	ND	**	
5 5246G	1	0.000	16.3	8.0	27.0	ND	ND	**	Recovery (%) = 101.9
6 5247G	1	0.000	16.4	7.9	27.0	ND	ND	**	Precision (RPD) = -0.68
7									
8									
9									
10									Sample volume (ml) 5.00
11									Dilution factor 1
12									
13									Sample Set Description:
14									Test No.:
15									Test Day:
16									Species:
17									
18									Sample Type (check):
19									Proj. No.: 865
20									Bulk sediment porewaters
21									Test beaker porewaters
22									X Overlying water
23									
24									Pre-purge analysis of overlying sulfide
25									in amphipod/worm beakers.
26									
27									Equals Day 0 overlying water in normal test.
28									
29									** Note: H ₂ S detection limit is 0.0008 mg/L as S
30									
31									
32									
33									
34									
35									
36									
37									
38									Analyst: RSC/JB
39									Date analysed: 3/25/2015
40									
* mg/L as sulfur									



Dissolved Sulfide in Water or Sediment Pore Water: Computation Worksheet									
Methylene Blue Method (SOP #5550)									
Standardization		1	2	3					
uL PAO titrant employed:		160	153	161					
Working Std. Conc. (mg/L):		3.2							
Result									
Sample description	Dilution factor	Temp (°C)	pH	Salinity (‰)	Total Soluble Sulfide (mg/L*)	H ₂ S (mg/L*)			
Blank	—	—	—	—	—	—			
1.0 mL working sulfide std.	—	140	—	—	0.64	—			
2.0 mL working sulfide std.	—	279	—	—	1.28	—			
3.0 mL working sulfide std.	—	440	—	—	1.92	—			
4.0 mL working sulfide std.	—	580	—	—	2.56	—			
5.0 mL working sulfide std.	—	710	—	—	3.20	—			
3.0 mL spike	—	437	—	—	—	—			
3.0 mL spike dupl.	—	440	—	—	—	—			
1	5.0 mL Smpl								
2	5230G	1	0.001			ND	**	Total Sulfide	
3	5231G	1	0.001			ND	**	Reporting limit (mg/l)	0.0200
4	5245G	1	0.000			ND	**		
5	5246G	1	0.000			ND	**	Recovery (%) =	#VALUE!
6	5247G	1	0.000			ND	**	Precision (RPD) =	#VALUE!
7									
8	** Note: H ₂ S detection limit is 0.0017 mg/L as S								
9									
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<div style="display: flex; justify-content: space-between;"> <div> <p>Sample volume (ml) 5.00</p> <p>Dilution factor 1</p> <p>Sample Set Description:</p> <p>Test No.: </p> <p>Test Day: </p> <p>Species: </p> <p>Sample Type (check):</p> <p>Proj. No.: 865</p> <p>Bulk sediment porewaters</p> <p>Test beaker porewaters</p> <p>X Overlying water</p> <p><u>Pre-purge analysis of overlying sulfide in amphipod/worm beakers.</u></p> <p><u>Equals Day 0 overlying water in normal test.</u></p> </div> <div> <p>Analyst: RSC/JB</p> <p>Date analysed: 3/25/2015</p> </div> </div>									

* mg/L as sulfur

Total Ammonia-N in Sediment Pore Water: Computation Worksheet Salicylate Method (SOP #5492)

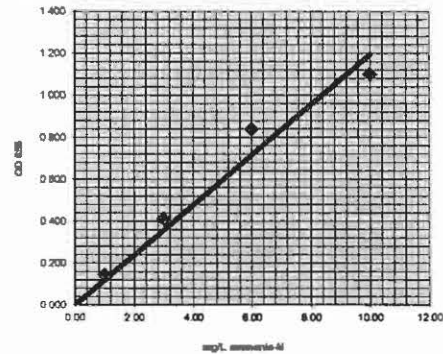
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449	3.71		
3.0 mg/L spike dupl.	----	0.444	3.67		
5.0 mg/L 2nd source		0.750	6.20		
1 5230G	5	0.189	7.81		
2 5231G	5	0.077	3.18		
3 5245G	5	0.283	11.70		
4 5246G	5	0.090	3.72		
5 5247G	5	0.025	1.03		

$$y = 0.1193x$$

$$R^2 = 0.9492$$

Standard Curve



Reporting limit (mg/L) = 0.50

Recovery (%) = 123.1

Precision (RPD) = 1.12

2nd source (%) = 124.0

Sample volume (ml): 0.10

Dilution factor 5

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)

X Bulk Sediment Porewaters

Test Beaker Porewaters

Overlying Water

Analyst:

Date analysed:

RSC/JB

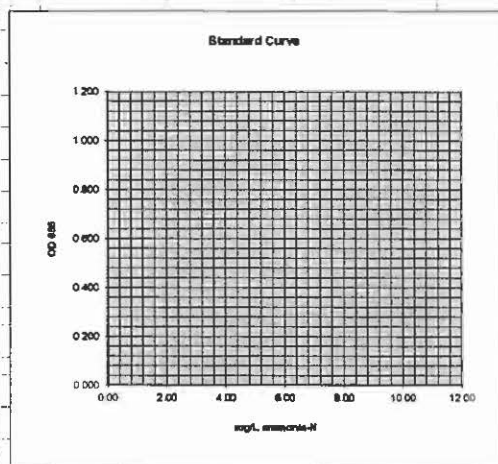
3/24/2015

Handwritten initials:
RSC
JB

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD655	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449			
3.0 mg/L spike dupl.	----	0.444			
5.0 mg/L 2nd source		0.750			
1 5230G	1	0.189		7.5	27.0
2 5231G	1	0.077		7.8	29.0
3 5245G	1	0.283		8.1	29.5
4 5246G	1	0.090		8.0	29.5
5 5247G	1	0.025		8.0	27.0



Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: P865

Test Day:

Species:


Sample Type (check)☒ Bulk Sediment Porewaters☐ Test Beaker Porewaters☐ Overlying Water

Analyst:

Date analysed:

RSC/JB

3/24/2015


 RSC/JB

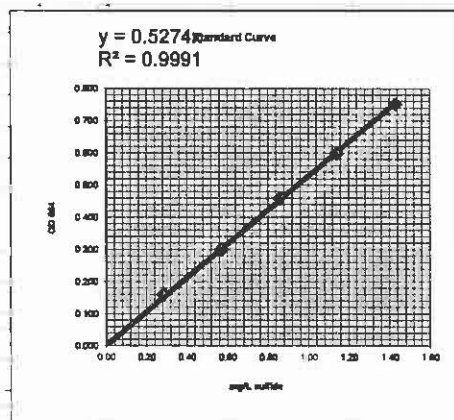
Dissolved Sulfide in Water: Computation Worksheet Methylene Blue Method (SOP #5550)

Standardization

	1	2	3
uL PAO titrant employed:	112	112	108
Working Std. Conc. (mg/L):		1.42933	

Result

Sample description	Dilution factor	OD664	Sulfide (mg/L)
Blank	---	---	---
1.0 mL working sulfide std.	---	0.161	0.29
2.0 mL working sulfide std.	---	0.300	0.57
3.0 mL working sulfide std.	---	0.460	0.86
4.0 mL working sulfide std.	---	0.599	1.14
5.0 mL working sulfide std.	---	0.751	1.43
3.0 mL spike	---	0.451	0.86
3.0 mL spike dupl.	---	0.450	0.85



1	5230G	5	0.025	0.24
2	5231G	5	0.021	0.20
3	5245G	5	0.005	ND
4	5246G	5	0.010	ND
5	5247G	5	0.000	ND
6				
7				
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36				

Reporting limit (mg/L) = 0.10

Recovery (%) = 99.6

Precision (RPD) = 0.22

Sample volume (ml): 1.00

Dilution factor 5

Sample Set Description:

Test No.:

Test Day:

Species:

Proj. No.: P865

X Bulk sediment porewaters

Test beaker porewaters

Overlying water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Dissolved Sulfide in Water: Computation Worksheet **Methylene Blue Method (SOP #5550)**

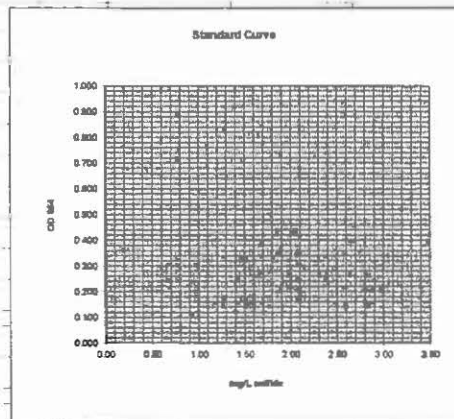
Standardization

uL PAO titrant employed:
 Working Std. Conc. (mg/L):

1	2	3
112	112	108
	3.2	

Result

Sample description	Dilution factor	OD664	Sulfide (mg/L)
Blank	-----	-----	-----
1.0 mL working sulfide std.	----	0.161	0.64
2.0 mL working sulfide std.	----	0.300	1.28
3.0 mL working sulfide std.	----	0.460	1.92
4.0 mL working sulfide std.	----	0.599	2.56
5.0 mL working sulfide std.	----	0.751	3.20
3.0 mL spike	----	0.451	
3.0 mL spike dupl.	----	0.450	



1	5230G	5	0.025
2	5231G	5	0.021
3	5245G	5	0.005
4	5246G	5	0.010
5	5247G	5	0.000
6			
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12			
13			
14			
15			
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33			
34			
35			
36			

Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

Sample volume (ml): 1.00

Dilution factor 5

Sample Set Description:

Test No.:

Test Day:

Species:

Proj. No.: P865

X Bulk sediment porewaters

Test beaker porewaters

Overlying water

Analyst:

Date analysed:

RSC/JB
3/24/2015

SECTION B

Amphipod (*Eohaustorius estuarius*) sediment bioassay 865-1 data report

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 865-1Title: *Eohaustorius estuarius* 10-day sediment toxicity test of marine sediments as part of Port of Seattle – West Waterway Deepening project.Protocol: NAS-XXX-EE4, February 20, 1992. Rev. 3 (March 1, 2005). Based on: Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995), with modifications as specified by the Dredged Material Management Program (DMMP, formerly Puget Sound Dredged Disposal Analysis Program or PSDDA). Dredged Material Evaluation and Disposal Procedures User Manual, December 2014.

STUDY MANAGEMENT

Study Sponsor: Anchor QEA, 720 Olive Way, Suite 1900, Seattle, Washington 98101.Sponsor's Study Monitor: Ms. Cindy FieldsTesting Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, Oregon 97365.Test Location: Newport Laboratory.Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Mngr./ Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, Ph.D., Sr. Aq. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; J. B. Brown, B.S., D.V.M., Assoc. Aq. Toxicol.; Y. Nakahama, Sr.Tech.; L. Brady, Tech.Study Schedule:

Test Beginning: 3-31-15, 1040 hrs.

Test Ending: 4-10-15, 1105 hrs.

Disposition of Study Records: All raw data, reports, and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Control Sediment: Control sediment (NAS Sample #5250G) was collected from the *Eohaustorius estuarius* amphipod collection site in lower Yaquina Bay, Oregon, on 3-25-15. The sediment was sieved through a 0.5-mm stainless steel screen and stored at 4°C in the dark.Test Sediments: Four test sediments and one reference sediment were tested. Details follow:

NAS Sample No.	5231G	5245G	5246G
Sample Description	WWD05-0-4-150206	WWD02-4-8-150205	WWD02-0-4-150205
Collection Date	2-6-15	2-5-15	2-5-15
Receipt Date	3-23-15	3-23-15	3-23-15
Interstitial Salinity (‰)	29.0	29.5	29.5

NAS Sample No.	5247G	5230G
Sample Description	WWD12-0-4-150206	WWD-REF-150319
Collection Date	2-6-15	3-19-15
Receipt Date	3-23-15	3-20-15
Interstitial Salinity (‰)	29.0	27.0

Storage: Samples were stored at 4°C in the dark.Treatments: The samples were minimally homogenized by mixing with stainless steel implements.

TEST WATER

Source: Yaquina Bay, OregonDate of Collection: 3-25-15Water Quality: Salinity 28.0‰; pH, 8.1Pretreatment: Filtered to ≤0.45 µm, salinity-adjusted with MilliQ® deionized water, aerated.

TEST ORGANISMS

Species: *Eohaustorius estuarius*, amphipod

Age: adult

Source: Yaquina Bay, Oregon

Acclimation: Amphipods were collected on 3-26-15 at interstitial water conditions of 12.0°C and 14.0‰. They were acclimated to test temperature and salinity over six days before addition to the test. Average holding conditions during this time were: temperature, $15.6 \pm 0.1^\circ\text{C}$; pH, 8.1 ± 0.2 ; salinity, $24.7 \pm 3.7\text{‰}$; and dissolved oxygen $8.3 \pm 0.2 \text{ mg/L}$. The photoperiod was constant light.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 1 L covered borosilicate glass beakers

Test Volumes: 175 ml of test, reference, or control sediment; 950 ml total volume.

Replicates/Treatment: 5 (plus one water quality replicate)

Salinity adjustment: none

Organisms/Treatment: 100 (20/replicate).

Water Volume Changes per 24 hr: None.

Aeration: Yes, at least 2 cm above the sediment surface.

Feeding: None.

Acceptance Criteria: Results are valid if mean control mortality does not exceed 10%, and does not exceed 20% in any one control replicate.

Performance Criteria: For testing under the Dredged Material Evaluation and Disposal Procedures User Manual, December 2014, the mean mortality in the reference sediment must not exceed the mean mortality in the control sediment by more than 20% absolute.

Effects Criteria: 1) mortality after 10 days, 2) daily emergence of amphipods from the test sediments, and 3) failure of surviving amphipods to rebury at the end of the exposure period. Death is defined as no visible appendage movement or response to tactile stimulation. Unrecovered animals at the end of the exposure period were considered dead.

Water Quality and Other Test Conditions: The temperature, dissolved oxygen, salinity, and pH, were measured in the water quality replicate test chamber daily. Total dissolved sulfide and total ammonia-N were measured in the overlying water of the water quality replicate test chamber on days 0 and 10. Interstitial total ammonia-N and total dissolved sulfide were measured in bulk sediments, and interstitial total ammonia-N was measured in the water quality beaker on test day 10. Interstitial water samples were obtained by centrifugation or by settling. Total soluble sulfide and total ammonia-N were measured using Hach reagents based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. Un-ionized ammonia-N was computed using "Un-ionized Ammonia Calculator", v1.0 (Dr. Landon Ross, Florida Department of Environmental Protection). The photoperiod was constant light.

DATA ANALYSIS METHODS

The percent amphipod mortality, percent of surviving amphipods failing to rebury at the end of the test, and percent total effective mortality were determined from the final observations according to the formulas:

$$\text{Percent Mortality} = 100 \times ([\text{initial amphipods} - \text{surviving amphipods}]/\text{initial amphipods})$$

$$\text{Percent Survivors not Reburied} = 100 \times ([\text{surviving amphipods} - \text{number survivors reburied}]/\text{surviving amphipods})$$

$$\text{Percent Total Effective Mortality} = 100 \times ([\text{initial amphipods} - \text{surviving amphipods}] + [\text{surviving amphipods} - \text{number survivors reburied}])/\text{initial amphipods}$$

Another endpoint was the sum of observed daily sediment emergence events in a test beaker throughout the test. Control and treatment means and standard deviations for the biological endpoints described above and for water quality data were computed using Microsoft EXCEL 2010. The software used for statistical comparisons

was BioStat (version Feb 9, 2006 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. Percent mortality in each test sediment was compared against that in the appropriate reference sediment. Generally, an arcsine square root transformation was performed on percentage data before analysis. In some cases, a rank order transformation was necessary. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.05 level of significance.

PROTOCOL DEVIATIONS

1. Several salinity measurements were above the protocol-specified range of $28.0 \pm 1.0\text{‰}$ (maximum 30.0‰).
2. The test water was held longer than the two days listed in the protocol. Rain forecasts prior to test start necessitated early test water collection.

REFERENCE TOXICANT TEST

The reference toxicant test is a standard multi-concentration toxicity test using ammonia expressed as $\text{NH}_3\text{-N}$ and administered as NH_3Cl , to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix II.

Test No.: 999-3406

Reference Toxicant and Source: Ammonia as ammonium chloride, Mallinckrodt, 10.0 mg/mL ammonia-as-N stock prepared 12-31-13.

Test Date: 3-31-15

Dilution Water Used: Yaquina Bay, Oregon, seawater; 28.0 ‰, pH 8.1

Result: The 96-hr LC_{50} was 225 mg/L $\text{NH}_3\text{-N}$. This result is within the laboratory's control chart warning limits (76.2–402 mg/L $\text{NH}_3\text{-N}$).

RESULTS AND DISCUSSION

Observations of overlying water quality parameters during the test are summarized in Table 1. Interstitial water quality measurements during the test are summarized in Table 2. Individual water quality measurements are located in the raw data (Appendix II).

Except as noted above, all measurements of standard water quality parameters were within protocol-specified ranges. Dissolved sulfide was not detected in the overlying water (detection limit 0.02 mg/L). Total ammonia-N concentrations in the overlying water ranged from <0.1 mg/L to 1.2 mg/L (maximum 0.038 mg/L un-ionized ammonia).

Interstitial total ammonia-N concentrations in the bulk sediment and beakers on day 10 ranged from 0.6 to 11.7 mg/L (maximum 0.385 mg/L un-ionized ammonia), with initial sulfide concentrations ranging from <0.1 to 0.2 mg/L.

Table 3 shows the effects of test sediment exposures on emergence, mortality, and reburial. The test met the acceptability criterion ($\leq 10\%$) for mean control mortality; mean mortality in the control was 1.0%. In addition, replicate control mortality was 0.0, 0.0, 0.0, 5.0, and 0.0%; therefore, the control replicate acceptability criterion was met ($\leq 20\%$ in any one replicate). The reference toxicant test result (225 mg/L $\text{NH}_3\text{-N}$) was within the laboratory's control chart warning limits. Mean mortality in reference sediment, WWD-REF-150319 (NAS #5230G) was 5.0%, well within the performance standard of not exceeding the mean mortality in the control sediment by more than 20%.

The test control acceptance criteria and the reference sediment performance criterion were met, and the positive control performance was within the laboratory's acceptance limits. It is concluded, therefore, that the test has developed acceptable data for use in making management decisions.

Test sediment WWD12-0-4-150206 was compared to the control sediment due to grain size considerations. All other test sediments were compared to the reference WWD-REF-150319. The percent mortality in test sediments

WWD02-0-4-150205 and WWD05-0-4-150206 was significantly higher ($P=0.05$) than that in the reference sediment.

Interpretation was based on guidelines from the "Dredged Material Evaluation and Disposal Procedures User Manual," December 2014. For a test sediment to fail under these guidelines, under the single hit rule, the mean test mortality must be $>20\%$ absolute over the mean negative control response, and $>10\%$ (dispersive) or $>30\%$ (nondispersive) absolute over the mean reference sediment response, and statistically different ($\alpha = 0.05$) from the reference sediment. Under these rules, two test sediments, WWD02-0-4-150205 and WWD05-0-4-150206 failed under the single hit dispersive rule (Table 4).

STUDY APPROVAL

Sheldahl Lusiani 6/3/15
Project Manager/ Study Director Date

Julie R. Fiere 6-3-15
Quality Assurance Unit Date

Linda K. Nemeth 6/2/15
Assistant Laboratory Director Date

Table 1. Summary of overlying water quality conditions during tests of the amphipod, *Eohaustorius estuarius*, exposed to marine sediments.

Parameter	Mean \pm SD	Minimum	Maximum	N
Temperature ($^{\circ}$ C)	15.2 \pm 0.3	14.8	16.1	66
pH	8.2 \pm 0.1	7.9	8.5	66
Salinity (‰)	28.9 \pm 0.7	27.0	30.0	66
Dissolved oxygen (mg/L)	7.8 \pm 0.2	6.5	8.1	67
Total soluble sulfide (mg/L)	---	<0.02	<0.02	12
Total ammonia-N (mg/L)	---	<0.1	1.2	12
Un-ionized ammonia (mg/L)	---	<0.003	0.038	12

Table 2. Summary of interstitial water quality conditions during tests of the amphipod, *Eohaustorius estuarius*, exposed to marine sediments.

Parameter	Mean \pm SD	Minimum	Maximum	N
Salinity (‰)	29.1 \pm 0.7	27.0	29.5	11
pH	7.7 \pm 0.3	6.9	8.1	11
Total soluble sulfide (mg/L)	---	<0.1	0.2	5
Total ammonia-N (mg/L)	---	0.6	11.7	11
Un-ionized ammonia (mg/L)	---	0.003	0.385	11

Table 3. Means and standard deviations (n=5) of sediment emergence, percent mortality, percent of survivors failing to rebury, and percent total effective mortality of *Eohaustorius estuarius* exposed to estuarine sediments.

Sample description	Emergence ¹ (no./replicate)	Percent mortality	Percent Survivors Failing to Rebury	Percent Total Effective Mortality
Control (NAS# 5250G)	0.0 \pm 0.0	1.0 \pm 2.2	0.0 \pm 0.0	1.0 \pm 2.2
WWD02-0-4-150205 (NAS# 5246G)	2.4 \pm 2.9	27.0 \pm 7.6*	0.0 \pm 0.0	27.0 \pm 7.6
WWD02-4-8-150205 (NAS# 5245G)	1.6 \pm 1.8	7.0 \pm 2.7	0.0 \pm 0.0	7.0 \pm 2.7
WWD05-0-4-150206 (NAS# 5231G)	3.2 \pm 2.4	25.0 \pm 11.7*	0.0 \pm 0.0	25.0 \pm 11.7
WWD12-0-4-150206 (NAS# 5247G) ²	0.0 \pm 0.0	1.0 \pm 2.2	0.0 \pm 0.0	1.0 \pm 2.2
WWD-REF-150319 (NAS# 5230G)	1.2 \pm 1.3	5.0 \pm 3.5	0.0 \pm 0.0	5.0 \pm 3.5

¹ Daily emergence counts include all amphipods observed on or above the sediment surface, whether living or dead.² Compared to the control sediment per client request.

*Percent mortality was significantly higher than that in reference sediment (p<0.05).

Table 4. Single-hit criteria interpretation of *Eohaustorius* test data.

Sample description	Percent mortality	Significantly different from the reference at $\alpha = 0.05$?	Percent difference over negative control	Percent difference over the reference	Failure under 1-hit dispersive rule?	Failure under 1-hit nondispersive rule?
Control (NAS# 5250G)	1.0 ± 2.2	---	---	---	---	---
WWD02-0-4-150205 (NAS# 5246G)	27.0 ± 7.6	Yes	26.0	22.0	Yes	No
WWD02-4-8-150205 (NAS# 5245G)	7.0 ± 2.7	No	6.0	2.0	No	No
WWD05-0-4-150206 (NAS# 5231G)	25.0 ± 11.7	Yes	24.0	20.0	Yes	No
WWD12-0-4-150206 ¹ (NAS# 5247G)	1.0 ± 2.2	No	0.0	-4.0	No	No
WWD-REF-150319 (NAS# 5230G)	5.0 ± 3.5	---	---	---	---	---
<p>If the mean test sediment mortality >20% absolute over the mean negative control response, and >10% (dispersive) or >30% (nondispersive) absolute over the mean reference sediment response, and statistically different ($\alpha = 0.05$) from the reference sediment, it fails under the single-hit rule.</p> <p>¹ Compared to the control sediment.</p>						

APPENDIX I
PROTOCOL

TEST PROTOCOL

**AMPHIPOD, *EOHAUSTORIUS ESTUARIUS*,
10-DAY SOLID PHASE SEDIMENT BIOASSAY**

1. **INTRODUCTION**

- 1.1 **Purpose of Study:** The purpose of this study is to identify marine test sediments that are toxic to an estuarine amphipod.
- 1.2 **Summary of Method:** The 10-day static test is performed using adult amphipods obtained from a wild population. Test sediments are placed in the bottom of 1-liter glass beakers used as test vessels that are then filled with clean seawater. Five replicate containers for each test sediment, reference site sediment, and the collection site control sediment, each containing 20 test organisms, are employed. Survival, emergence from the sediment during exposure, and failure to rebury in clean sediment after the test are the response criteria used. The mean and standard deviation for each treatment and test endpoint are given in the final report. Between-treatment statistical comparisons may be made, where each treatment is compared to the control and/or reference sediment. This protocol is based on PSEP (1995) guidelines, PSDDA (PSDDA 1989, U.S. ACOE et al. 2000) modifications, SMS (WDOE 2003) and ASTM E-1367 (ASTM 2004).

2. **STUDY MANAGEMENT**

2.1 **Sponsor's Name and Address:**

2.2 **Sponsor's Study Monitor:**

2.3 **Name of Testing Laboratory:**

Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365

2.4 **Test Location:** Newport Laboratory

2.5 **Laboratory's Personnel to be Assigned to the Study:**

Project Manager/Technical Director: _____
Quality Assurance Officer: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 **Proposed Testing Schedule:** Tests should begin within 2 weeks (8 weeks with samples under nitrogen for PSDDA or SMS) of sample collection. A reference toxicant test is run concurrently.

2.7 **Good Laboratory Practices:** The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. **TEST MATERIAL**

The test materials are marine or estuarine sediments. *E. estuarius* is a desirable test species for sediments with interstitial water salinity of 2-28 ppt, and the use of the *E. estuarius* bioassay is preferred for sediments with salinities <25 ppt. The collected sediments are placed in a suitable container for shipping and storage. The preferred container is a solvent and acid cleaned 1 L glass jar fitted with a TFE-lined screw cap. The jars are filled completely so that there is no air space. At the laboratory, the samples may be stored at 4°C in the dark in the original sealed containers for up to 2 weeks (8 weeks with no headspace or with samples under nitrogen for PSDDA

or SMS) prior to testing. The negative control sediment is from a clean site, normally the amphipod collection site. In addition, one or more reference sediments, clean sediments with physical characteristics similar to the test sediments, may also be employed.

4. TEST WATER

Test water is filtered Yaquina Bay seawater adjusted to the selected test salinity. The water is pumped from Yaquina Bay into a 6000 gal seasoned fiberglass reservoir from which it is supplied under pump pressure to the laboratory. Filtration is accomplished using a sand filter followed by 5 μm , 1 μm , and 0.40 μm cartridge filters. An alternative seawater supply of similar quality may be used. Seawater should be held at $\leq 15^{\circ}\text{C}$ for ≤ 2 days prior to test initiation.

5. TEST ORGANISMS

5.1 Species: Estuarine amphipod, *Eohaustorius estuarius*.

5.2 Source: Field collected from the lower portion of Beaver Creek, OR, or Yaquina Bay, OR in the intertidal zone. Interstitial water salinity and temperature are measured at the collection site. The sediments are sieved in the field using a 1.0 mm screen and the recovered amphipods, along with miscellaneous debris, are washed into plastic pails of collection site sediment and returned promptly to the laboratory.

5.3 Laboratory Handling: Pails containing the amphipods are placed in a laboratory water bath or controlled temperature room for temperature control and supplied with gentle aeration. As soon as possible after collection, the amphipods are sieved from the pails of sediment and are removed from the holding vessel using a fine mesh aquarium dip net and placed into Pyrex glass sorting trays. The test organisms are picked from the detritus using a large bore eye dropper and 100 amphipods each are placed into small plastic freezer containers (9 cm x 9 cm x 6 cm) holding a 1 cm layer of clean sediment from the collection area. Freezer containers are immersed in a seawater tray provided with seawater and aeration, and held under constant illumination for at least 2 days, but no longer than 10 days prior to the beginning of the test. It may be necessary to acclimate the amphipods to the test salinity, depending upon the collection site salinity and the test water salinity. Although moderate rates of salinity change are preferred, this species is tolerant of rapid salinity changes (DeWitt, et al., 1989).

5.4 Age at Study Initiation: Adult

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 1000 ml glass beakers. The beakers are covered to minimize contamination and evaporation of seawater or loss of volatile compounds. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Minimal aeration (approx. 100 bubbles/min.) is supplied through a glass pipet with the tip placed at least 2 cm above the sediment surface. The test is performed under continuous illumination, using ambient laboratory lighting. SMS may require UV light for PAH-contaminated intertidal sediments.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in an automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times more with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: An experimental design is used consisting of exposure of test organisms to a number of test sediments, one or more reference sediments, and to the collection site control sediment. Each treatment consists of five replicate test chambers each containing 20 animals. An additional replicate containing 20 test organisms is used for daily water quality measurements. More replicates, with or without

test organisms as appropriate, may be employed for periodic interstitial water quality measurements. Blind, random testing is used.

- 7.2 Preparation of Test Sediments: The interstitial salinity of the test, reference, and control sediments is measured. Adjustment of interstitial salinities is not recommended for *Eohaustorius estuarius* under standard guidelines, as *E. estuarius* tolerates a broad salinity range and any adjustments disturb the test sediments. However, if client project specifications require interstitial salinity adjustment, the PSEP (1995) procedure described for *Rhepoxynius abronius* and *Neanthes arenaceodentata* testing may be used.

Certain projects may require purging of excess ammonia from sediment interstitial water. PSDDA and SMS allow purging to be considered when interstitial total ammonia concentrations are 60 mg/L or above (at pH = 7.7; un-ionized >0.8 mg/L). However, purging is allowed only by agency permission, so the decision to purge should be made by the client. Testing of purged sediments may require concurrent testing of the same sediments unpurged.

If no salinity adjustment or purging is done, sediments are used without further treatment. Each test sediment is mixed thoroughly using a non-contaminating implement, then an aliquot (175 ml) sufficient to make a 2-cm-deep layer is added to each test beaker, and the surface is smoothed. Bubbles are removed from the sediment by gently tapping each beaker against the palm of the hand. Seawater at the test temperature and salinity is carefully added into the beaker to the 750 ml mark utilizing a water dispersal technique to avoid suspending the sediment. The beakers are then placed into the water bath or constant-temperature room and covered with watchglasses. An air delivery pipet is inserted into each beaker under the watchglass. Overhead lights provide constant illumination. Water in the test beakers is aerated without disturbing the sediments. The test system is then allowed to temperature equilibrate overnight.

- 7.3 Beginning the Test: Amphipods are wet sieved, using a 1.0 mm sieve, from the holding sediment and impartially distributed to a series of seawater-filled containers each holding 10 amphipods. Two containers of 10 animals each are randomly added to each replicate. Once amphipods are added to a replicate, the number of animals that do not burrow into the test sediment within 15 minutes is recorded. Amphipods not burrowed are removed and replaced with healthy amphipods, unless they are actively swimming or burrowing and re-emerging, as this may be a response to toxic material. Following addition of amphipods to the test chambers, additional water is added to achieve a final volume of 950 ml.
- 7.4 Effects Criteria: Effects criteria are 1) survival after 10 days, 2) daily emergence of amphipods from the test sediments, and 3) failure of amphipods to rebury in sediment at the end of the exposure period. Death is defined as no visible appendage movement or response to tactile stimulation.
- 7.5 Test Conditions: Test containers are maintained at a constant $15 \pm 1^\circ\text{C}$. The selected test salinity is kept within ± 1 ppt. Frequently, a test salinity of 28 ppt is used to be consistent with *Rhepoxynius* and *Ampelisca* tests. The dissolved oxygen concentration in each test container must be greater than 60% saturation throughout the 10-day test. Each beaker is supplied with oil-free compressed air provided at a rate of approximately 100 bubbles per minute through disposable glass pipettes positioned with their tips at least 2 cm above the sediment surface. Each beaker is covered by a watchglass to minimize evaporation and the possibility of cross contamination between beakers. The test is conducted under constant illumination.
- 7.6 Feeding: Animals are not fed at any time before or during the test.
- 7.7 Test Duration, Type and Frequency of Observations, and Methods: The duration of the sediment toxicity test is 10 days. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION
Biological Data	
Emergence from sediment	daily
Survival	end of test
Reburial	end of test
Physical and Chemical Data	
Sediment interstitial salinity	at test beginning (bulk sediments) & end
Salinity, dissolved oxygen, pH, & temperature of overlying water (1 replicate only)	daily
Ammonia and sulfides in overlying water (1 replicate)	at test beginning & end
Ammonia, sulfide, pH, & salinity in interstitial water (optional)	for PSDDA and SMS, ammonia, pH, & salinity in bulk sediments and at test beginning and end; sulfide as requested by client
Check air and lights	daily

The presence of amphipods that have emerged from the sediments is recorded daily. Any other unusual observations are recorded. No amphipods are removed at any time until the termination of the test. The bioassay is terminated after 10 days of sediment exposure. The sediment is wet sieved through a 0.5 mm screen to recover buried amphipods. The number of surviving amphipods is recorded. For the reburial endpoint, surviving amphipods from each beaker are transferred to containers with a layer of control sediment and observed under constant illumination. The numbers of amphipods able to bury within a 1-hour exposure period are then recorded.

Dissolved oxygen is measured directly in test beakers using an air-calibrated polarographic oxygen probe. The pH is measured using a properly calibrated pH meter with scale divisions of 0.1 pH units. Temperature is measured using a calibrated mercury thermometer or a telethermometer. Salinity is measured using a refractometer. The method used for the measurement of total ammonia-N in the overlying water and sediment porewater from sediment bioassays is based on the salicylate colorimetric method of Hach Chemical Co. and was adapted from Clin. Chim. Acta., 14: 403 (1966). The method used for the measurement of dissolved sulfide in the overlying water and sediment porewater in marine sediment bioassays is the methylene blue colorimetric method based on SM 4500-S²⁻ (Standard Methods 1995 (19th edition). Any observed changes in sediment color or the formation of a sediment discontinuity layer is also recorded.

7.8 Criteria of Test Acceptance: For the test to be considered acceptable, the minimum mean survival of organisms in the control treatment at the end of the test must be 90%, and survival in each control replicate must be at least 80%.

7.9 Performance Criterion: For PSDDA, mean percent mortality in the reference sediments must be $\leq 20\%$ over the negative control. For SMS, mean percent mortality in the reference sediments must be $< 25\%$.

7.10 Reference Toxicant Test: A routine reference toxicant test is run concurrently with each sediment test. This may be a 48-hr test with sodium dodecyl sulfate (preferred), or a 96-hr test with cadmium chloride (frequently required by client). PSDDA and SMS also require a water-only ammonia test when any test sediment interstitial ammonia is > 30 mg/L total ammonia (at pH=7.7; un-ionized > 0.4 mg/L).

8. DATA ANALYSIS

The mean and standard deviation are calculated for each endpoint employed (e.g. mortality) and for each treatment (i.e. test sediment). Between-treatment comparisons may be made using a t-test (Student's t-test, approximate t-test, one-sample t-test), Wilcoxon Two-Sample test, Mann-Whitney test, or Rankit Analysis, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons. An estimate of

total effective mortality (optional) may also be calculated by summing percent mortality and percent failure to rebury.

9. REPORTING

A report of the test results must include the following information: name and identification of the test; the investigator and laboratory; sediment holding temperature data; information on the test sediment including the interstitial salinity for control, reference and test sediments; information on the source of seawater used; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including water quality; information about any aeration that may have been required; definition of the effects criteria and other observations; unusual responses, if any, in the control treatment; daily emergence for each beaker and the 10-day mean and S.D. for each treatment; 10-day mortality for each beaker and the mean and S.D. for each treatment; failure to rebury and total effective mortality (optional) in each beaker and the mean and S.D. for each treatment; 96-hr LC50 with reference toxicant; a description of data analysis methods employed and documentation of statistical test results; any unusual information about the test or deviations from procedures.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect, and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCES FOR PROCEDURES

ASTM. 2004. Standard test method for measuring the toxicity of sediment-associated contaminants with estuarine and marine invertebrates. ASTM Standard Method No. E1367-03e1. In: 2004 Annual Book of ASTM Standards, volume 11.05, Biological effects and environmental fate; biotechnology; pesticides. ASTM International, West Conshohocken, PA.

DeWitt, T.H., R.C. Swartz and J.O. Lamberson. 1989. Measuring the acute toxicity of estuarine sediments. Environ. Toxicol. Chem. 8: 1035-1048.

Puget Sound Dredged Disposal Analysis (PSDDA). 1989. Management plan report - unconfined open-water disposal of dredged material, Phase II - (north and south Puget Sound). Puget Sound Dredged Disposal Analysis, Army Corps of Engineers, Seattle, WA. (and other modifications made through the PSDDA process and Sediment Management Annual Review Meetings).

Puget Sound Estuary Program. 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound sediments. Prepared for U.S. Env. Prot. Agency, Region 10, Office of Puget Sound, Seattle, WA and Puget Sound Water Quality Authority, P.O. Box 40900, Olympia, WA.

U.S. Army Corps of Engineers, Seattle District; U.S. EPA, Region 10; Washington Department of Natural Resources; Washington Department of Ecology. 2000. Dredged material evaluation and disposal procedures: a user's manual for the dredged disposal analysis (PSDDA) program.

Washington State Department of Ecology. 2003. Sediment sampling and analysis plan appendix. Publication no. 03-09-043, WDOE, Olympia, WA.

Weber, C.I. (Ed.) 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Fourth Edition). EPA/600/4-90/027F.

12. APPROVALS

_____	_____	for _____
Name	Date	
_____	_____	for NORTHWESTERN AQUATIC SCIENCES
Name	Date	

APPENDIX II

RAW DATA

**TEST DESCRIPTION, MONITORING, AND RESULTS
BENCHSHEETS**

NORTHWESTERN AQUATIC SCIENCES
MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

PROTOCOL NO. NAS-XXX-

REVIEWED
PAGES 1-474
-651

Test No. 865-1 Client Anchor QEA Investigator

STUDY MANAGEMENT

Client: ANCHOR QEA, LLC, 720 Olive Way, Suite 1900, Seattle, WA 98101

Client's Study Monitor: Ms. Cindy Fields

Testing Laboratory: Northwestern Aquatic Sciences

Test Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Man./Study Dir. G.J. Irissarri ⁶⁵¹

QA Officer L.K. Nemeth

1. Yves Nataguma ✓

2. GA Buhler OB

3. Lauren Brady LB

4. J. Brown JS

5.

6. D.J. Caldwell PSC

7.

8.

Study Schedule:

Test Beginning: 3-31-15 1040

Test Ending: 4-10-15 1105

TEST MATERIAL

General description (see sample logbook/chain-of-custody for details):

NAS Sample No.:	5231G	5245G	5246G	5247G	5230G
Description:	WWD05-0-4-150206	WWD02-4-8-150205	WWD02-0-4-150205	WWD12-0-4-150206	WWD-REF-150319
Collection Date:	2/6/2015	2/5/2015	2/5/2015	2/6/2015	3/19/2015
Receipt Date:	3/23/2015	3/23/2015	3/23/2015	3/23/2015	3/20/2015
Inters.Salinity (ppt):	29.0	29.5	29.5	29.0	27.0
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					

Error codes: 1) correction of handwriting error

2) written in wrong location; entry deleted

3) wrong date deleted, replaced with correct date

4) error found in measurement; measurement repeated Page 1 of 47

Test No. 865-1 Client Anchor QEA Investigator _____

SEDIMENT DESCRIPTIONS -- SUPPLEMENTAL NOTES

[illegible]

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

TEST ORGANISMS

Species: Eohaustorius estuarius Date Collected: 3-26-15
Source: Yaquina Bay, OR

Field conditions when collected:

Interstitial temperature: 12.0°C Interstitial salinity: 14.0 ppt

Acclimation Data:

Date	Temp. (deg.C)	pH	Sal (ppt)	DO (mg/L)	Feeding		Water changes	Comments
					amount	description		
3-26-15	15.6	7.79	20.0	8.6	NOT	FED	YES	
3-27-15	15.5	8.0	20.0	8.3			YES	
3-28-15	15.6	8.1	26.5	8.1			-	
3-29-15	15.5	8.1	26.5	8.2			YES	
3-30-15	15.6	8.1	28.0	8.2			-	
3-31-15	15.7	8.1	27.0	8.2			-	
Mean	15.6	8.0	24.7	8.3				
S.D.	0.1	0.2	3.7	0.2				
(N)	6	6	6	6				

Photoperiod during acclimation: CONSTANT LIGHT

TEST PROCEDURES AND CONDITIONS

Test chambers: 1 L glass beakers covered with watchglasses

Test volumes: 175 ml of test sediment; 950 ml total volume

Replicates/treatment: (5) 5 Organisms/treatment: (100) 100 (20/REP)

Additional replicates included for water quality purposes (indicate numbers of each that apply):

☒ Water quality beaker (with test organisms) (may also be Day 10 sacrificial beaker)☐ Day zero sacrificial beaker (no test organisms)☐ Ammonia-purging sacrificial beakers (no test organisms)☐ Day _____ sacrificial beaker(s) (with test organisms)

Test water changes: None

Feeding: None

Beaker placement: Total randomization

Aeration: Yes, at least 2 cm above the sediment surface

Photoperiod: Constant light

Test temperature (deg.C): 15 ± 1.0Salinity (ppt): 28.0 ± 1.0

MISCELLANEOUS NOTES

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client Anchor QEA Investigator _____

Control Sediment:

Source: Yaquina Bay, OR
 Date collected: 3/25/15 Interstitial salinity: 22.0 ppt
 Sieved through 0.5 -mm screen
 Storage: 4°C in the dark NAS# 5250G

Test conducted in (circle one): room 1 room 2 room 3 room 4 trailer water bath

Randomization chart:

TOP SHELF

3									36
2									35
1									34

FRONT

Randomization chart:

Randomization chart:

Randomization chart:

TEST WATER

Source: Yaquina Bay, Oregon, sea water
 Date of Collection: 3-25-15 Salinity (ppt) 28.0 pH 8.1
 Treatments: filtered to 0.4 um, salinity-adjusted with MilliQ deionized water, aerated
0.45 um
5-13-15

Test No. 865-1 Client Anchor QEA Investigator

DAILY RECORD SHEET

overlying NH₃ sampled (days 0,10)? γ_e

Day 0 (3 131 15') LB/LB

[illegible]

* Water Quality Beakers

** Emergence is measured every day. However, for day zero only, this is number not buried within 10-15 minutes

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 1 (4/1/15) 05

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	ru	
2					0	ru	
3					0		
4					0		
5					0		
*6	15-2	8.2	27.5	8.0	0		
*7	15-4	8.1	28.0	8.1	0		
8					0		
9					0		
*10	15-3	8.1	28.0	7.9	0		
11					0		
12					0		
13					0		
14					0		
15					0		
16					0		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					0		
25					0		
*26	15-2	8.1	28.0	7.6	0		
*27	15-2	8.1	27.5	7.8	0		
28					0		
29					0		
30					0		
31					0		
*32	15-1	8.1	28.0	7.7	0		
33					0		
34					0		
35					1		
36					0	✓	

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 2 (4/2/15) W/H

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	OK	
2					0		
3					0		
4					0		
5					0		
*6	15.5	8.2	28.5	8.1	0		
*7	15.5	8.1	28.5	8.1	0		
8					1		
9					0		
*10	15.5	8.0	28.0	7.9	1		
11					0		
12					0		
13					0		
14					0		
15					0		
16					0		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					0		
25					0		
*26	15.4	8.1	28.5	7.9	0		
*27	15.3	8.0	29.0	7.7	2		
28					0		
29					0		
30					0		
31					0		
*32	15.2	8.1	28.5	7.9	0		
33					0		
34					0		
35					1		
36					0		

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 3 (4 13 115) 12/12

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	OK	
2					0		
3					0		
4					0		
5					0		
*6	15.2	8.2	29.0	8.0	0		
*7	15.4	8.1	29.0	7.9	0		
8					0		
9					0		
*10	15.4	8.1	29.0	7.9	0		
11					0		
12					0		
13					0		
14					0		
15					0		
16					0		
17					0		
18					0		
19					0		
20					1		
21					0		
22					0		
23					0		
24					0		
25					0		
*26	15.3	8.1	29.0	7.9	0		
*27	15.3	8.1	29.5	7.5	0		
28					0		
29					2		
30					0		
31					0		
*32	15.1	8.2	28.5	7.9	0		
33					0		
34					0		
35					0		
36					0		

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client Anchor QEA Investigator

DAILY RECORD SHEET

Day 4 (4/4/15) 047

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	Am	
2					0	OK	
3					0		
4					0		
5					0		
*6	15.0	8.2	29.0	7.8	0		
*7	15.1	8.1	29.0	7.9	0		
8					2		
9					0		
*10	15.1	8.1	29.5	8.1	1		
11					0		
12					0		
13					0		
14					0		
15					0		
16					1		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					2		
25					0		
*26	15.0	8.2	29.0	7.7	2		
*27	15.0	8.1	29.0	7.7	0		
28					0		
29					1		
30					0		
31					1		
*32	15.0	8.2	29.0	7.9	0		
33					0		
34					0		
35					0		
36					0	✓	

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 5 (4 / 5 / 15) 631

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	OK	
2					0		
3					0		
4					0		
5					0		
*6	14.9	8.3	29.0	7.9	0		
*7	15.1	8.1	29.5	7.9	0		
8					1		
9					0		
*10	15.1	8.2	29.5	7.8	1		
11					0		
12					0		
13					0		
14					2		
15					0		
16					1		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					0		
25					2		
*26	14.9	8.2	29.0	7.8	0		
*27	15.0	8.1	30.0	7.7	0		
28					0		
29					0		
30					0		
31					0		
*32	14.9	8.2	29.5	7.8	0		
33					0		
34					0		
35					0		
36					0	7	

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 6 (4 / 6 / 15) US

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1	14.8	8.4	28.4	8.0	0	OK	
2				USDO 4.6	0		
3					0		
4					0		
5					0		
*6	14.8	8.4	29.0	8.0	0		
*7	15.1	8.2	28.5	7.6	0		
8					0		
9					0		
*10	15.1	8.3	28.5	7.8	1		
11					0		
12					0		
13					0		
14					1		
15					0		
16					0		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					0		
25					0		
*26	15.0	8.2	29.0	8.0	0		
*27	14.9	8.2	29.5	7.9	1		
28					0		
29					0		
30					0		
31					0		
*32	14.9	8.3	29.0	7.8	0		
33					0		
34					0		
35					1		
36					0		

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 7 (4/7/15)

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	OK	
2					0		
3					0		
4					0		
5					0		
*6	15.0	8.4	29.5	8.0	0		
*7	15.1	8.2	29.0	7.9	0		
8					0		
9					0		
*10	15.1	8.3	29.0	7.9	1		
11					0		
12					0		
13					0		
14					1		
15					2		
16					0		
17					1		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					0		
25					0		
*26	15.1	8.2	29.5	7.9	0		
*27	15.0	8.2	29.5	7.9	1		
28					0		
29					0		
30					0		
31					0		
*32	14.9	8.3	29.5	8.1	0		
33					0		
34					0		
35					0		
36					0	↓	

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 8 (4 / 8 / 15) 43

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	ON	
2					0		
3					0		
4					0		
5					0		
*6	15.2	8.4	29.0	7.9	0		
*7	15.3	8.1	28.5	7.9	0		
8					0		
9					0		
*10	15.3	8.3	29.0	7.8	0		
11					0		
12					0		
13					0		
14					1		
15					0		
16					3		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					1		
25					1		
*26	15.2	8.1	29.5	7.8	0	↓	
*27	15.2	8.2	30.0	7.0	0	OFF	all DO 6.5 + need air on
28					0	OK	
29					0		
30					0		
31					1		
*32	15.1	8.2	30.0	7.4	0		
33					0		
34					0		
35					1		
36					0	↓	

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 9 (419115)[✓]

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	No. emerged	Air	Comments
1					0	0/C	
2					0		
3					1		
4					0		
5					0		
*6	15.3	8.4	29.5	7.9	0		
*7	15.3	8.1	29.5	7.9	0		
8					0		
9					0		
*10	15.3	8.3	29.5	7.7	1		
11					0		
12					0		
13					0		
14					0		
15					0		
16					1		
17					0		
18					0		
19					0		
20					0		
21					0		
22					0		
23					0		
24					0		
25					1		
*26	15.2	8.1	29.5	7.7	0		
*27	15.2	8.2	29.0	7.8	0		
28					0		
29					0		
30					0		
31					1		
*32	15.1	8.2	29.5	7.9	0		
33					0		
34					0		
35					2		
36					0	✓	

* Water Quality Beakers

MARINE AMPHIPOD 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-1 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

overlying NH₃ sampled (days 0,10)? yesDay 10 (4 / 10 / 15) 07

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	S (mg/L)	No. emerged	Air	Comments
1						0	ALL	
2						1	OXYGEN	
3						1		
4						0		
5						0		
*6	15.1	8.5	29.5	7.8	10.02	0		
*7	15.1	8.1	30.0	7.9	10.02	0		
8						0		
9						0		
*10	15.2	8.4	29.5	7.7	10.02	0		
11						0		
12						0		
13						0		
14						0		
15						0		
16						0		
17						1		
18						0		
19						0		
20						0		
21						0		
22						0		
23						1		
24						0		
25						1		
*26	15.0	8.1	29.5	7.8	10.02	0		
*27	15.0	8.3	29.5	7.8	10.02	0		
28						0		
29						0		
30						0		
31						0		
*32	14.9	8.2	30.0	7.9	10.02	0		
33						0		
34						0		
35						0		
36						0	↓	

* Water Quality Beakers

Test No. 865-1 Client Anchor QEA Investigator

DAY 10 TEST TERMINATION SHEET

Note: reburial is not an endpoint for the *Ampelisca* test

[illegible]

 water quality or day zero beakers

TEST DATA ANALYSIS RECORDS

Endpoints Data Entry and Calculations File

INIT=initial number
SURV=number survivors
REBUR=number survivors which reburied
MORT=number dead=INIT-SURV
NOBURY=number survivors not reburied=SURV-REBUR
TEM=total effective mortality=MORT+NOBURY

PSURV=%survival=100(SURV/INIT)
PMORT=%mortality=100(MORT/INIT)
PBURY=%reburial=100(REBUR/SURV)
PNOBURY=%survivors not reburied=100(NO BURY/SURV)
PTM=%total effective mortality=100(TEM/INIT)

IN-DEX	NAS-BKR	CLIENT-SMPL	CLIENT-DESCRIP	REPL	INIT	SURV	REBUR	MORT	NO-BURY	TEM	PSURV	PMORT	PBURY	PNO-BURY	PTEM		SURV	MORT	NO-BURY	PSURV	PMORT	PBURY	PNO-BURY	PTEM
1	4	5250G	Control	1	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0									
2	11	5250G	Control	2	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0	Mean	19.8	0.2	0.0	99.0	1.0	100.0	0.0	1.0
3	22	5250G	Control	3	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0	SD	0.4	0.4	0.0	2.2	2.2	0.0	0.0	2.2
4	21	5250G	Control	4	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	n	5	5	5	5	5	5	5	5
5	15	5250G	Control	5	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0									
6	32	5250G	Control	6 wq repl	20																			
7	30	5246G	WWD02-0-4-150205	1	20	15	15	5	0	5	75.0	25.0	100.0	0.0	25.0									
8	25	5246G	WWD02-0-4-150205	2	20	16	16	4	0	4	80.0	20.0	100.0	0.0	20.0	Mean	14.6	5.4	0.0	73.0	27.0	100.0	0.0	27.0
9	13	5246G	WWD02-0-4-150205	3	20	13	13	7	0	7	65.0	35.0	100.0	0.0	35.0	SD	1.5	1.5	0.0	7.6	7.6	0.0	0.0	7.6
10	20	5246G	WWD02-0-4-150205	4	20	13	13	7	0	7	65.0	35.0	100.0	0.0	35.0	n	5	5	5	5	5	5	5	5
11	16	5246G	WWD02-0-4-150205	5	20	16	16	4	0	4	80.0	20.0	100.0	0.0	20.0									
12	27	5246G	WWD02-0-4-150205	6 wq repl	20																			
13	8	5245G	WWD02-4-8-150205	1	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0									
14	24	5245G	WWD02-4-8-150205	2	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	Mean	18.6	1.4	0.0	93.0	7.0	100.0	0.0	7.0
15	19	5245G	WWD02-4-8-150205	3	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	SD	0.5	0.5	0.0	2.7	2.7	0.0	0.0	2.7
16	9	5245G	WWD02-4-8-150205	4	20	18	18	2	0	2	90.0	10.0	100.0	0.0	10.0	n	5	5	5	5	5	5	5	5
17	23	5245G	WWD02-4-8-150205	5	20	18	18	2	0	2	90.0	10.0	100.0	0.0	10.0									
18	26	5245G	WWD02-4-8-150205	6 wq repl	20																			
19	14	5231G	WWD05-0-4-150206	1	20	16	16	4	0	4	80.0	20.0	100.0	0.0	20.0									
20	17	5231G	WWD05-0-4-150206	2	20	16	16	4	0	4	80.0	20.0	100.0	0.0	20.0	Mean	15.0	5.0	0.0	75.0	25.0	100.0	0.0	25.0
21	28	5231G	WWD05-0-4-150206	3	20	11	11	9	0	9	55.0	45.0	100.0	0.0	45.0	SD	2.3	2.3	0.0	11.7	11.7	0.0	0.0	11.7
22	31	5231G	WWD05-0-4-150206	4	20	17	17	3	0	3	85.0	15.0	100.0	0.0	15.0	n	5	5	5	5	5	5	5	5
23	35	5231G	WWD05-0-4-150206	5	20	15	15	5	0	5	75.0	25.0	100.0	0.0	25.0									
24	10	5231G	WWD05-0-4-150206	6 wq repl	20																			
25	33	5247G	WWD12-0-4-150206	1	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0									
26	18	5247G	WWD12-0-4-150206	2	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0	Mean	19.8	0.2	0.0	99.0	1.0	100.0	0.0	1.0
27	5	5247G	WWD12-0-4-150206	3	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	SD	0.4	0.4	0.0	2.2	2.2	0.0	0.0	2.2
28	12	5247G	WWD12-0-4-150206	4	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0	n	5	5	5	5	5	5	5	5
29	36	5247G	WWD12-0-4-150206	5	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0									
30	7	5247G	WWD12-0-4-150206	6 wq repl	20																			
31	3	5230G	WWD-REF-150319	1	20	20	20	0	0	0	100.0	0.0	100.0	0.0	0.0									
32	2	5230G	WWD-REF-150319	2	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	Mean	19.0	1.0	0.0	95.0	5.0	100.0	0.0	5.0
33	34	5230G	WWD-REF-150319	3	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	SD	0.7	0.7	0.0	3.5	3.5	0.0	0.0	3.5
34	29	5230G	WWD-REF-150319	4	20	19	19	1	0	1	95.0	5.0	100.0	0.0	5.0	n	5	5	5	5	5	5	5	5
35	1	5230G	WWD-REF-150319	5	20	18	18	2	0	2	90.0	10.0	100.0	0.0	10.0									
36	6	5230G	WWD-REF-150319	6 wq repl	20																			

Project Name: P865-1 Eohaustorius % Mortality

Sample: x1
 Samp ID: WWD02-0-4-150205
 Alias: NAS# 5246G
 Replicates: 5
 Mean: 27
 SD: 7.583
 Tr Mean: 31.134
 Trans SD: 4.894

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 5
 SD: 3.536
 Tr Mean: 11.44
 Trans SD: 6.826

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 3.854 SS: 282.201 K: 5 b: 16.172 Alpha Level: 0.05 Calculated Value: 0.9268 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 4.109 Test Residual SD: 1.687 Ref. Residual Mean: 4.576 Ref. Residual SD: 4.519 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.2162 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: ArcSin Experimental Hypothesis Null: $x1 \leq x2$ Alternate: $x1 > x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 5.2431 Critical Value: ≥ 1.860 Accept Null Hypothesis: No Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	25	30	0	0	1.134	11.44			-11.44
2	20	26.565	5	12.921	4.569	1.481			-4.569
3	35	36.271	5	12.921	5.137	1.481			-4.569
4	35	36.271	5	12.921	5.137	1.481			-1.134
5	20	26.565	10	18.435	4.569	6.995			1.481
6									1.481
7									1.481
8									5.137
9									5.137
10									6.995

The percent mortality in test sediment WWD02-0-4-150205 was significantly higher than that of the reference sediment (WWD-REF-150319) at $\alpha=0.05$.

-631

Project Name: P865-1 Eohaustorius % Mortality

Sample: x1
 Samp ID: WWD02-4-8-150205
 Alias: NAS# 5245G
 Replicates: 5
 Mean: 7
 SD: 2.739
 Tr Mean: 15.127
 Trans SD: 3.02

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 5
 SD: 3.536
 Tr Mean: 11.44
 Trans SD: 6.826

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 3.425 SS: 222.867 K: 5 b: 14.026 Alpha Level: 0.05 Calculated Value: 0.8828 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 2.647 Test Residual SD: 0.604 Ref. Residual Mean: 4.576 Ref. Residual SD: 4.519 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.9461 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: ArcSin Experimental Hypothesis Null: $x1 \leq x2$ Alternate: $x1 > x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 1.1045 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	5	12.921	0	0	2.206	11.44			-11.44
2	5	12.921	5	12.921	2.206	1.481			-2.206
3	5	12.921	5	12.921	2.206	1.481			-2.206
4	10	18.435	5	12.921	3.308	1.481			-2.206
5	10	18.435	10	18.435	3.308	6.995			1.481
6									1.481
7									1.481
8									3.308
9									3.308
10									6.995

The percent mortality in test sediment WWD02-4-8-150205 was not significantly higher than that of the reference sediment (WWD-REF-150319) at $\alpha=0.05$.

Project Name: P865-1 Eohaustorius % Mortality

Sample: x1
 Samp ID: WWD05-0-4-150206
 Alias: NAS# 5231G
 Replicates: 5
 Mean: 25
 SD: 11.726
 Tr Mean: 29.609
 Trans SD: 7.45

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 5
 SD: 3.536
 Tr Mean: 11.44
 Trans SD: 6.826

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 4.636 SS: 408.399 K: 5 b: 19.865 Alpha Level: 0.05 Calculated Value: 0.9663 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 5.165 Test Residual SD: 4.708 Ref. Residual Mean: 4.576 Ref. Residual SD: 4.519 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.2018 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: ArcSin Experimental Hypothesis Null: $x1 \leq x2$ Alternate: $x1 > x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: 4.0209 Critical Value: ≥ 1.860 Accept Null Hypothesis: No Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	20	26.565	0	0	3.044	11.44			-11.44
2	20	26.565	5	12.921	3.044	1.481			-6.823
3	45	42.13	5	12.921	12.521	1.481			-3.044
4	15	22.786	5	12.921	6.823	1.481			-3.044
5	25	30	10	18.435	0.391	6.995			0.391
6									1.481
7									1.481
8									1.481
9									6.995
10									12.521

The percent mortality in test sediment WWD05-0-4-150206 was significantly higher than that of the reference sediment (WWD-REF-150319) at $\alpha=0.05$.

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Project Name: P865-1 Eohaustorius % Mortality

Sample: x1
 Samp ID: WWD12-0-4-150206
 Alias: NAS# 5247G
 Replicates: 5
 Mean: 1
 SD: 2.236
 Tr Mean: N/A
 Trans SD: N/A

Ref Samp: x2
 Ref ID: Control
 Alias: NAS# 5250G
 Replicates: 5
 Mean: 1
 SD: 2.236
 Tr Mean: N/A
 Trans SD: N/A

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 3.75 SS: 267.122 K: 5 b: 11.668 Alpha Level: 0.05 Calculated Value: 0.5096 Critical Value: ≤ 0.842 Normally Distributed: No Override Option: Not Invoked	Test Residual Mean: 4.135 Test Residual SD: 3.467 Ref. Residual Mean: 4.135 Ref. Residual SD: 3.467 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Mann-Whitney Balanced Design: Yes Transformation: rank-order Experimental Hypothesis Null: $x1 \leq x2$ Alternate: $x1 > x2$ Mann-Whitney N1: 5 Mann-Whitney N2: 5 Degrees of Freedom: Experimental Alpha Level: 0.05 Calculated Value: 12.5 Critical Value: ≥ 21.000 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0	4.5	0	4.5	2.584	2.584	4.5		-2.584
2	0	4.5	0	4.5	2.584	2.584	4.5		-2.584
3	5	9.5	0	4.5	10.337	2.584	4.5		-2.584
4	0	4.5	5	9.5	2.584	10.337	4.5		-2.584
5	0	4.5	0	4.5	2.584	2.584	4.5		-2.584
6							4.5		-2.584
7							4.5		-2.584
8							4.5		-2.584
9							9.5		10.337
10							9.5		10.337

The percent mortality in test sediment WWD12-0-4-150206 was not significantly higher than that of the control sediment at $\alpha=0.05$.

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Emergence Data File						
INDEX	BKR	NAS SMPL	CLIENT DESCRIP	REPL	DAY	EMERG EMERG
1	4	5250G	Control	1	1	0
1	4	5250G	Control	1	2	0
1	4	5250G	Control	1	3	0
1	4	5250G	Control	1	4	0
1	4	5250G	Control	1	5	0
1	4	5250G	Control	1	6	0
1	4	5250G	Control	1	7	0
1	4	5250G	Control	1	8	0
1	4	5250G	Control	1	9	0
1	4	5250G	Control	1	10	0
2	11	5250G	Control	2	1	0
2	11	5250G	Control	2	2	0
2	11	5250G	Control	2	3	0
2	11	5250G	Control	2	4	0
2	11	5250G	Control	2	5	0
2	11	5250G	Control	2	6	0
2	11	5250G	Control	2	7	0
2	11	5250G	Control	2	8	0
2	11	5250G	Control	2	9	0
2	11	5250G	Control	2	10	0
3	22	5250G	Control	3	1	0
3	22	5250G	Control	3	2	0
3	22	5250G	Control	3	3	0
3	22	5250G	Control	3	4	0
3	22	5250G	Control	3	5	0
3	22	5250G	Control	3	6	0
3	22	5250G	Control	3	7	0
3	22	5250G	Control	3	8	0
3	22	5250G	Control	3	9	0
3	22	5250G	Control	3	10	0
4	21	5250G	Control	4	1	0
4	21	5250G	Control	4	2	0
4	21	5250G	Control	4	3	0
4	21	5250G	Control	4	4	0
4	21	5250G	Control	4	5	0
4	21	5250G	Control	4	6	0
4	21	5250G	Control	4	7	0
4	21	5250G	Control	4	8	0
4	21	5250G	Control	4	9	0
4	21	5250G	Control	4	10	0
5	15	5250G	Control	5	1	0
5	15	5250G	Control	5	2	0
5	15	5250G	Control	5	3	0
5	15	5250G	Control	5	4	0
5	15	5250G	Control	5	5	0
5	15	5250G	Control	5	6	0
5	15	5250G	Control	5	7	0
5	15	5250G	Control	5	8	0
Mean						0.0
SD						0.0
n						5

data entry verified
against laboratory
bench sheets 5-13-15
JRF

5	15	5250G	Control	5	9	0		
5	15	5250G	Control	5	10	0	0	
7	3	5230G	WWD-REF-150319	1	1	0		
7	3	5230G	WWD-REF-150319	1	2	0		
7	3	5230G	WWD-REF-150319	1	3	0		
7	3	5230G	WWD-REF-150319	1	4	0		
7	3	5230G	WWD-REF-150319	1	5	0		
7	3	5230G	WWD-REF-150319	1	6	0		
7	3	5230G	WWD-REF-150319	1	7	0		
7	3	5230G	WWD-REF-150319	1	8	0		
7	3	5230G	WWD-REF-150319	1	9	1		
7	3	5230G	WWD-REF-150319	1	10	1	2	
8	2	5230G	WWD-REF-150319	2	1	0		
8	2	5230G	WWD-REF-150319	2	2	0		
8	2	5230G	WWD-REF-150319	2	3	0		
8	2	5230G	WWD-REF-150319	2	4	0		
8	2	5230G	WWD-REF-150319	2	5	0		
8	2	5230G	WWD-REF-150319	2	6	0		
8	2	5230G	WWD-REF-150319	2	7	0		
8	2	5230G	WWD-REF-150319	2	8	0		
8	2	5230G	WWD-REF-150319	2	9	0		
8	2	5230G	WWD-REF-150319	2	10	1	1	
9	34	5230G	WWD-REF-150319	3	1	0		
9	34	5230G	WWD-REF-150319	3	2	0		
9	34	5230G	WWD-REF-150319	3	3	0		
9	34	5230G	WWD-REF-150319	3	4	0		
9	34	5230G	WWD-REF-150319	3	5	0		
9	34	5230G	WWD-REF-150319	3	6	0		
9	34	5230G	WWD-REF-150319	3	7	0		
9	34	5230G	WWD-REF-150319	3	8	0		
9	34	5230G	WWD-REF-150319	3	9	0		
9	34	5230G	WWD-REF-150319	3	10	0	0	
10	29	5230G	WWD-REF-150319	4	1	0		
10	29	5230G	WWD-REF-150319	4	2	0		
10	29	5230G	WWD-REF-150319	4	3	2		
10	29	5230G	WWD-REF-150319	4	4	1		
10	29	5230G	WWD-REF-150319	4	5	0		
10	29	5230G	WWD-REF-150319	4	6	0		
10	29	5230G	WWD-REF-150319	4	7	0		
10	29	5230G	WWD-REF-150319	4	8	0		
10	29	5230G	WWD-REF-150319	4	9	0		
10	29	5230G	WWD-REF-150319	4	10	0	3	
11	1	5230G	WWD-REF-150319	5	1	0		
11	1	5230G	WWD-REF-150319	5	2	0		
11	1	5230G	WWD-REF-150319	5	3	0	Mean	1.2
11	1	5230G	WWD-REF-150319	5	4	0	SD	1.3
11	1	5230G	WWD-REF-150319	5	5	0	n	5
11	1	5230G	WWD-REF-150319	5	6	0		
11	1	5230G	WWD-REF-150319	5	7	0		
11	1	5230G	WWD-REF-150319	5	8	0		
11	1	5230G	WWD-REF-150319	5	9	0		
11	1	5230G	WWD-REF-150319	5	10	0	0	

13	14	5231G	WWD05-0-4-150206	1	1	0		
13	14	5231G	WWD05-0-4-150206	1	2	0		
13	14	5231G	WWD05-0-4-150206	1	3	0		
13	14	5231G	WWD05-0-4-150206	1	4	0		
13	14	5231G	WWD05-0-4-150206	1	5	2		
13	14	5231G	WWD05-0-4-150206	1	6	1		
13	14	5231G	WWD05-0-4-150206	1	7	1		
13	14	5231G	WWD05-0-4-150206	1	8	1		
13	14	5231G	WWD05-0-4-150206	1	9	0		
13	14	5231G	WWD05-0-4-150206	1	10	0	5	
14	17	5231G	WWD05-0-4-150206	2	1	0		
14	17	5231G	WWD05-0-4-150206	2	2	0		
14	17	5231G	WWD05-0-4-150206	2	3	0		
14	17	5231G	WWD05-0-4-150206	2	4	0		
14	17	5231G	WWD05-0-4-150206	2	5	0		
14	17	5231G	WWD05-0-4-150206	2	6	0		
14	17	5231G	WWD05-0-4-150206	2	7	1		
14	17	5231G	WWD05-0-4-150206	2	8	0		
14	17	5231G	WWD05-0-4-150206	2	9	0		
14	17	5231G	WWD05-0-4-150206	2	10	1	2	
15	28	5231G	WWD05-0-4-150206	3	1	0		
15	28	5231G	WWD05-0-4-150206	3	2	0		
15	28	5231G	WWD05-0-4-150206	3	3	0		
15	28	5231G	WWD05-0-4-150206	3	4	0		
15	28	5231G	WWD05-0-4-150206	3	5	0		
15	28	5231G	WWD05-0-4-150206	3	6	0		
15	28	5231G	WWD05-0-4-150206	3	7	0		
15	28	5231G	WWD05-0-4-150206	3	8	0		
15	28	5231G	WWD05-0-4-150206	3	9	0		
15	28	5231G	WWD05-0-4-150206	3	10	0	0	
16	31	5231G	WWD05-0-4-150206	4	1	0		
16	31	5231G	WWD05-0-4-150206	4	2	0		
16	31	5231G	WWD05-0-4-150206	4	3	0		
16	31	5231G	WWD05-0-4-150206	4	4	1		
16	31	5231G	WWD05-0-4-150206	4	5	0		
16	31	5231G	WWD05-0-4-150206	4	6	0		
16	31	5231G	WWD05-0-4-150206	4	7	0		
16	31	5231G	WWD05-0-4-150206	4	8	1		
16	31	5231G	WWD05-0-4-150206	4	9	1		
16	31	5231G	WWD05-0-4-150206	4	10	0	3	
17	35	5231G	WWD05-0-4-150206	5	1	1		
17	35	5231G	WWD05-0-4-150206	5	2	1		
17	35	5231G	WWD05-0-4-150206	5	3	0	Mean	3.2
17	35	5231G	WWD05-0-4-150206	5	4	0	SD	2.4
17	35	5231G	WWD05-0-4-150206	5	5	0	n	5
17	35	5231G	WWD05-0-4-150206	5	6	1		
17	35	5231G	WWD05-0-4-150206	5	7	0		
17	35	5231G	WWD05-0-4-150206	5	8	1		
17	35	5231G	WWD05-0-4-150206	5	9	2		
17	35	5231G	WWD05-0-4-150206	5	10	0	6	
19	8	5245G	WWD02-4-8-150205	1	1	0		
19	8	5245G	WWD02-4-8-150205	1	2	1		

19	8	5245G	WWD02-4-8-150205	1	3	0		
19	8	5245G	WWD02-4-8-150205	1	4	2		
19	8	5245G	WWD02-4-8-150205	1	5	1		
19	8	5245G	WWD02-4-8-150205	1	6	0		
19	8	5245G	WWD02-4-8-150205	1	7	0		
19	8	5245G	WWD02-4-8-150205	1	8	0		
19	8	5245G	WWD02-4-8-150205	1	9	0		
19	8	5245G	WWD02-4-8-150205	1	10	0	4	
20	24	5245G	WWD02-4-8-150205	2	1	0		
20	24	5245G	WWD02-4-8-150205	2	2	0		
20	24	5245G	WWD02-4-8-150205	2	3	0		
20	24	5245G	WWD02-4-8-150205	2	4	2		
20	24	5245G	WWD02-4-8-150205	2	5	0		
20	24	5245G	WWD02-4-8-150205	2	6	0		
20	24	5245G	WWD02-4-8-150205	2	7	0		
20	24	5245G	WWD02-4-8-150205	2	8	1		
20	24	5245G	WWD02-4-8-150205	2	9	0		
20	24	5245G	WWD02-4-8-150205	2	10	0	3	
21	19	5245G	WWD02-4-8-150205	3	1	0		
21	19	5245G	WWD02-4-8-150205	3	2	0		
21	19	5245G	WWD02-4-8-150205	3	3	0		
21	19	5245G	WWD02-4-8-150205	3	4	0		
21	19	5245G	WWD02-4-8-150205	3	5	0		
21	19	5245G	WWD02-4-8-150205	3	6	0		
21	19	5245G	WWD02-4-8-150205	3	7	0		
21	19	5245G	WWD02-4-8-150205	3	8	0		
21	19	5245G	WWD02-4-8-150205	3	9	0		
21	19	5245G	WWD02-4-8-150205	3	10	0	0	
22	9	5245G	WWD02-4-8-150205	4	1	0		
22	9	5245G	WWD02-4-8-150205	4	2	0		
22	9	5245G	WWD02-4-8-150205	4	3	0		
22	9	5245G	WWD02-4-8-150205	4	4	0		
22	9	5245G	WWD02-4-8-150205	4	5	0		
22	9	5245G	WWD02-4-8-150205	4	6	0		
22	9	5245G	WWD02-4-8-150205	4	7	0		
22	9	5245G	WWD02-4-8-150205	4	8	0		
22	9	5245G	WWD02-4-8-150205	4	9	0		
22	9	5245G	WWD02-4-8-150205	4	10	0	0	
23	23	5245G	WWD02-4-8-150205	5	1	0		
23	23	5245G	WWD02-4-8-150205	5	2	0		
23	23	5245G	WWD02-4-8-150205	5	3	0	Mean	1.6
23	23	5245G	WWD02-4-8-150205	5	4	0	SD	1.8
23	23	5245G	WWD02-4-8-150205	5	5	0	n	5
23	23	5245G	WWD02-4-8-150205	5	6	0		
23	23	5245G	WWD02-4-8-150205	5	7	0		
23	23	5245G	WWD02-4-8-150205	5	8	0		
23	23	5245G	WWD02-4-8-150205	5	9	0		
23	23	5245G	WWD02-4-8-150205	5	10	1	1	
25	30	5246G	WWD02-0-4-150205	1	1	0		
25	30	5246G	WWD02-0-4-150205	1	2	0		
25	30	5246G	WWD02-0-4-150205	1	3	0		
25	30	5246G	WWD02-0-4-150205	1	4	0		

25	30	5246G	WWD02-0-4-150205	1	5	0		
25	30	5246G	WWD02-0-4-150205	1	6	0		
25	30	5246G	WWD02-0-4-150205	1	7	0		
25	30	5246G	WWD02-0-4-150205	1	8	0		
25	30	5246G	WWD02-0-4-150205	1	9	0		
25	30	5246G	WWD02-0-4-150205	1	10	0	0	
26	25	5246G	WWD02-0-4-150205	2	1	0		
26	25	5246G	WWD02-0-4-150205	2	2	0		
26	25	5246G	WWD02-0-4-150205	2	3	0		
26	25	5246G	WWD02-0-4-150205	2	4	0		
26	25	5246G	WWD02-0-4-150205	2	5	2		
26	25	5246G	WWD02-0-4-150205	2	6	0		
26	25	5246G	WWD02-0-4-150205	2	7	0		
26	25	5246G	WWD02-0-4-150205	2	8	1		
26	25	5246G	WWD02-0-4-150205	2	9	1		
26	25	5246G	WWD02-0-4-150205	2	10	1	5	
27	13	5246G	WWD02-0-4-150205	3	1	0		
27	13	5246G	WWD02-0-4-150205	3	2	0		
27	13	5246G	WWD02-0-4-150205	3	3	0		
27	13	5246G	WWD02-0-4-150205	3	4	0		
27	13	5246G	WWD02-0-4-150205	3	5	0		
27	13	5246G	WWD02-0-4-150205	3	6	0		
27	13	5246G	WWD02-0-4-150205	3	7	0		
27	13	5246G	WWD02-0-4-150205	3	8	0		
27	13	5246G	WWD02-0-4-150205	3	9	0		
27	13	5246G	WWD02-0-4-150205	3	10	0	0	
28	20	5246G	WWD02-0-4-150205	4	1	0		
28	20	5246G	WWD02-0-4-150205	4	2	0		
28	20	5246G	WWD02-0-4-150205	4	3	1		
28	20	5246G	WWD02-0-4-150205	4	4	0		
28	20	5246G	WWD02-0-4-150205	4	5	0		
28	20	5246G	WWD02-0-4-150205	4	6	0		
28	20	5246G	WWD02-0-4-150205	4	7	0		
28	20	5246G	WWD02-0-4-150205	4	8	0		
28	20	5246G	WWD02-0-4-150205	4	9	0		
28	20	5246G	WWD02-0-4-150205	4	10	0	1	
29	16	5246G	WWD02-0-4-150205	5	1	0		
29	16	5246G	WWD02-0-4-150205	5	2	0		
29	16	5246G	WWD02-0-4-150205	5	3	0	Mean	2.4
29	16	5246G	WWD02-0-4-150205	5	4	1	SD	2.9
29	16	5246G	WWD02-0-4-150205	5	5	1	n	5
29	16	5246G	WWD02-0-4-150205	5	6	0		
29	16	5246G	WWD02-0-4-150205	5	7	0		
29	16	5246G	WWD02-0-4-150205	5	8	3		
29	16	5246G	WWD02-0-4-150205	5	9	1		
29	16	5246G	WWD02-0-4-150205	5	10	0	6	
31	33	5247G	WWD12-0-4-150206	1	1	0		
31	33	5247G	WWD12-0-4-150206	1	2	0		
31	33	5247G	WWD12-0-4-150206	1	3	0		
31	33	5247G	WWD12-0-4-150206	1	4	0		
31	33	5247G	WWD12-0-4-150206	1	5	0		
31	33	5247G	WWD12-0-4-150206	1	6	0		

31	33	5247G	WWD12-0-4-150206	1	7	0		
31	33	5247G	WWD12-0-4-150206	1	8	0		
31	33	5247G	WWD12-0-4-150206	1	9	0		
31	33	5247G	WWD12-0-4-150206	1	10	0	0	
32	18	5247G	WWD12-0-4-150206	2	1	0		
32	18	5247G	WWD12-0-4-150206	2	2	0		
32	18	5247G	WWD12-0-4-150206	2	3	0		
32	18	5247G	WWD12-0-4-150206	2	4	0		
32	18	5247G	WWD12-0-4-150206	2	5	0		
32	18	5247G	WWD12-0-4-150206	2	6	0		
32	18	5247G	WWD12-0-4-150206	2	7	0		
32	18	5247G	WWD12-0-4-150206	2	8	0		
32	18	5247G	WWD12-0-4-150206	2	9	0		
32	18	5247G	WWD12-0-4-150206	2	10	0	0	
33	5	5247G	WWD12-0-4-150206	3	1	0		
33	5	5247G	WWD12-0-4-150206	3	2	0		
33	5	5247G	WWD12-0-4-150206	3	3	0		
33	5	5247G	WWD12-0-4-150206	3	4	0		
33	5	5247G	WWD12-0-4-150206	3	5	0		
33	5	5247G	WWD12-0-4-150206	3	6	0		
33	5	5247G	WWD12-0-4-150206	3	7	0		
33	5	5247G	WWD12-0-4-150206	3	8	0		
33	5	5247G	WWD12-0-4-150206	3	9	0		
33	5	5247G	WWD12-0-4-150206	3	10	0	0	
34	12	5247G	WWD12-0-4-150206	4	1	0		
34	12	5247G	WWD12-0-4-150206	4	2	0		
34	12	5247G	WWD12-0-4-150206	4	3	0		
34	12	5247G	WWD12-0-4-150206	4	4	0		
34	12	5247G	WWD12-0-4-150206	4	5	0		
34	12	5247G	WWD12-0-4-150206	4	6	0		
34	12	5247G	WWD12-0-4-150206	4	7	0		
34	12	5247G	WWD12-0-4-150206	4	8	0		
34	12	5247G	WWD12-0-4-150206	4	9	0		
34	12	5247G	WWD12-0-4-150206	4	10	0	0	
35	36	5247G	WWD12-0-4-150206	5	1	0		
35	36	5247G	WWD12-0-4-150206	5	2	0		
35	36	5247G	WWD12-0-4-150206	5	3	0	Mean	0.0
35	36	5247G	WWD12-0-4-150206	5	4	0	SD	0.0
35	36	5247G	WWD12-0-4-150206	5	5	0	n	5
35	36	5247G	WWD12-0-4-150206	5	6	0		
35	36	5247G	WWD12-0-4-150206	5	7	0		
35	36	5247G	WWD12-0-4-150206	5	8	0		
35	36	5247G	WWD12-0-4-150206	5	9	0		
35	36	5247G	WWD12-0-4-150206	5	10	0	0	

Water Quality Data																				assumed	
NAS		CLIENT	Overlying water										Interstitial					interstitial			
BKR	SMPL	DESCRIP	REPL	DAY	TEMP	pH	SAL	DO	S	< or >	NH4+NH3	< or >	NH3	pH	SAL	Su#ide	< or >	NH4+NH3	< or >	NH3	temp
Bulk	5230G	WWD-REF-150319												7.5	27.0	0.2		7.8		0.087	15
Bulk	5231G	WWD05-0-4-150206												7.8	29.0	0.2		3.2		0.053	
Bulk	5245G	WWD02-4-8-150205												8.1	29.5	<0.1		11.7		0.385	
Bulk	5246G	WWD02-0-4-150205												8.0	29.5	<0.1		3.7		0.098	
Bulk	5247G	WWD12-0-4-150206												8.0	29.0	<0.1		1.0		0.027	
6	5230G	WWD-REF-150319	6	0	16.0	8.0	27.0	8.0	<0.02		0.7		0.019								
7	5247G	WWD12-0-4-150206	6	0	15.8	8.0	28.0	8.0	<0.02	<	0.1	<	0.003								
10	5231G	WWD05-0-4-150206	6	0	16.1	7.9	28.0	7.4	<0.02		0.1		0.003								
26	5245G	WWD02-4-8-150205	6	0	15.7	8.1	28.0	7.7	<0.02		0.5		0.016								
27	5246G	WWD02-0-4-150205	6	0	15.5	8.0	28.0	7.6	<0.02		0.2		0.005								
32	5250G	Control	6	0	15.5	8.1	28.0	8.1	<0.02	<	0.1	<	0.003								
6	5230G	WWD-REF-150319	6	1	15.2	8.2	27.5	8.0													
7	5247G	WWD12-0-4-150206	6	1	15.4	8.1	28.0	8.1													
10	5231G	WWD05-0-4-150206	6	1	15.3	8.1	28.0	7.9													
26	5245G	WWD02-4-8-150205	6	1	15.2	8.1	28.0	7.8													
27	5246G	WWD02-0-4-150205	6	1	15.2	8.1	27.5	7.8													
32	5250G	Control	6	1	15.1	8.1	28.0	7.7													
6	5230G	WWD-REF-150319	6	2	15.5	8.2	28.5	8.1													
7	5247G	WWD12-0-4-150206	6	2	15.5	8.1	28.5	8.1													
10	5231G	WWD05-0-4-150206	6	2	15.5	8.0	28.0	7.9													
26	5245G	WWD02-4-8-150205	6	2	15.4	8.1	28.5	7.9													
27	5246G	WWD02-0-4-150205	6	2	15.3	8.0	29.0	7.7													
32	5250G	Control	6	2	15.2	8.1	28.5	7.9													
6	5230G	WWD-REF-150319	6	3	15.2	8.2	29.0	8.0													
7	5247G	WWD12-0-4-150206	6	3	15.4	8.1	29.0	7.9													
10	5231G	WWD05-0-4-150206	6	3	15.4	8.1	29.0	7.9													
26	5245G	WWD02-4-8-150205	6	3	15.3	8.1	29.0	7.9													
27	5246G	WWD02-0-4-150205	6	3	15.3	8.1	29.5	7.5													
32	5250G	Control	6	3	15.1	8.2	28.5	7.9													
6	5230G	WWD-REF-150319	6	4	15.0	8.2	29.0	7.8													
7	5247G	WWD12-0-4-150206	6	4	15.1	8.1	29.0	7.9													
10	5231G	WWD05-0-4-150206	6	4	15.1	8.1	29.5	8.1													
26	5245G	WWD02-4-8-150205	6	4	15.0	8.2	29.0	7.7													
27	5246G	WWD02-0-4-150205	6	4	15.0	8.1	29.0	7.7													
32	5250G	Control	6	4	15.0	8.2	29.0	7.9													
6	5230G	WWD-REF-150319	6	5	14.9	8.3	29.0	7.9													
7	5247G	WWD12-0-4-150206	6	5	15.1	8.1	29.5	7.9													
10	5231G	WWD05-0-4-150206	6	5	15.1	8.2	29.5	7.8													
26	5245G	WWD02-4-8-150205	6	5	14.9	8.2	29.0	7.8													
27	5246G	WWD02-0-4-150205	6	5	15.0	8.1	30.0	7.7													
32	5250G	Control	6	5	14.9	8.2	29.5	7.8													
6	5230G	WWD-REF-150319	6	6	14.8	8.4	29.0	8.0													
7	5247G	WWD12-0-4-150206	6	6	15.1	8.2	28.5	7.8													
10	5231G	WWD05-0-4-150206	6	6	15.1	8.3	28.5	7.8													
26	5245G	WWD02-4-8-150205	6	6	15.0	8.2	29.0	8.0													
27	5246G	WWD02-0-4-150205	6	6	14.9	8.2	29.5	7.9													
32	5250G	Control	6	6	14.9	8.3	29.0	7.8													
6	5230G	WWD-REF-150319	6	7	15.0	8.4	29.5	8.0													
7	5247G	WWD12-0-4-150206	6	7	15.1	8.2	29.0	7.9													
10	5231G	WWD05-0-4-150206	6	7	15.1	8.3	29.0	7.9													
26	5245G	WWD02-4-8-150205	6	7	15.1	8.2	29.5	7.9													
27	5246G	WWD02-0-4-150205	6	7	15.0	8.2	29.5	7.9													
32	5250G	Control	6	7	14.9	8.3	29.5	8.1													
6	5230G	WWD-REF-150319	6	8	15.2	8.4	29.0	7.9													
7	5247G	WWD12-0-4-150206	6	8	15.3	8.1	28.5	7.9													
10	5231G	WWD05-0-4-150206	6	8	15.3	8.3	29.0	7.8													
26	5245G	WWD02-4-8-150205	6	8	15.2	8.1	29.5	7.8													
27	5246G	WWD02-0-4-150205	6	8	15.2	8.2	30.0	6.5													
32	5250G	Control	6	8	15.1	8.2	30.0	7.0													
6	5230G	WWD-REF-150319	6	8	15.1	8.2	30.0	7.9													
7	5247G	WWD12-0-4-150206	6	9	15.3	8.4	29.5	7.9													
10	5231G	WWD05-0-4-150206	6	9	15.3	8.1	29.5	7.9													
26	5245G	WWD02-4-8-150205	6	9	15.3	8.3	29.5	7.7													
27	5246G	WWD02-0-4-150205	6	9	15.2	8.1	29.5	7.7													
32	5250G	Control	6	9	15.2	8.2	29.0	7.8													
6	5230G	WWD-REF-150319	6	9	15.1	8.2	29.5	7.9													
7	5247G	WWD12-0-4-150206	6	10	15.1	8.5	29.5	7.8	<0.02	<	0.1	<	0.008	6.9	29.5			1.4		0.003	
10	5231G	WWD05-0-4-150206	6	10	15.1	8.1	30.0	7.9	<0.02	<	0.1	<	0.003	7.5	29.5			0.6		0.005	
26	5245G	WWD02-4-8-150205	6	10	15.2	8.4	29.5	7.7	<0.02		0.1		0.008	7.7	29.0			0.6		0.008	
27	5246G	WWD02-0-4-150205	6	10	15.0	8.1	29.5	7.8	<0.02		1.2		0.038	7.9	29.0			3.1		0.085	
32	5250G	Control	6	10	15.0	8.3	29.5	7.8	<0.02		0.1		0.007	7.7	29.5			1.1		0.014	
6	5230G	WWD-REF-150319	6	10	14.9	8.2	30.0	7.9	<0.02	<	0.1	<	0.004	7.6	29.5			0.8		0.008	
Mean					15.2	8.2	28.9	7.8	—	—	—	—	—	7.7	29.1	—	—	—	—	—	—
SD					0.3	0.1	0.7	0.2	—	—	—	—	—	0.3	0.7	—	—	—	—	—	—
n					66	66	66	67	12	12	12	11	11	5	11	11	11	11	11	11	11
Min					14.8	7.9	27.0	6.5	<0.02	<0.1	<	0.003	6.9	27.0	<0.1	0.6		0.003		0.003	
Max					16.1	8.5	30.0	8.1	<0.02	1.2	0.038	8.1	29.5	0.2	11.7	0.385		0.385		0.385	

AMMONIA EXPOSURE BENCHSHEETS AND ANALYSIS

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

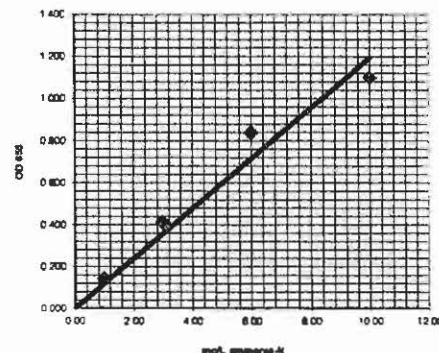
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	-----	-----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449	3.71		
3.0 mg/L spike dupl.	----	0.444	3.67		
5.0 mg/L 2nd source		0.750	6.20		
1 5230G	5	0.189	7.81		
2 5231G	5	0.077	3.18		
3 5245G	5	0.283	11.70		
4 5246G	5	0.090	3.72		
5 5247G	5	0.025	1.03		

$$y = 0.1193x$$

$$R^2 = 0.9492$$

Standard Curve



Reporting limit (mg/L) = 0.50

Recovery (%) = 123.1

Precision (RPD) = 1.12

2nd source (%) = 124.0

Sample volume (ml): 0.10

Dilution factor 5

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)

- X Bulk Sediment Porewaters
 Test Beaker Porewaters
 Overlying Water

Analyst:

Date analysed:

RSC/JB

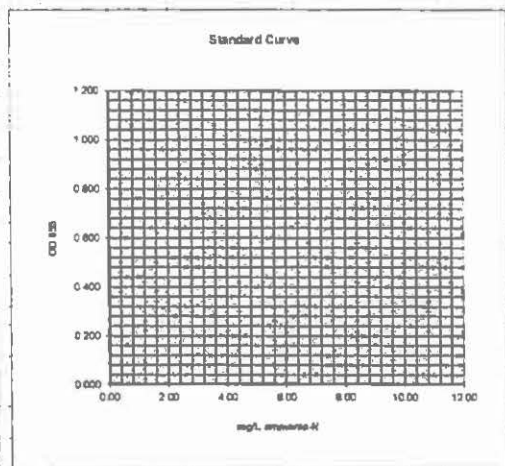
3/24/2015

Handwritten initials: RSC, JB

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	-----	-----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449			
3.0 mg/L spike dupl.	----	0.444			
5.0 mg/L 2nd source		0.750			
1 5230G	1	0.189		7.5	27.0
2 5231G	1	0.077		7.8	29.0
3 5245G	1	0.283		8.1	29.5
4 5246G	1	0.090		8.0	29.5
5 5247G	1	0.025		8.0	27.0



Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)☒ Bulk Sediment Porewaters☐ Test Beaker Porewaters☐ Overlying Water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Handwritten initials: RSC
Handwritten initials: JB

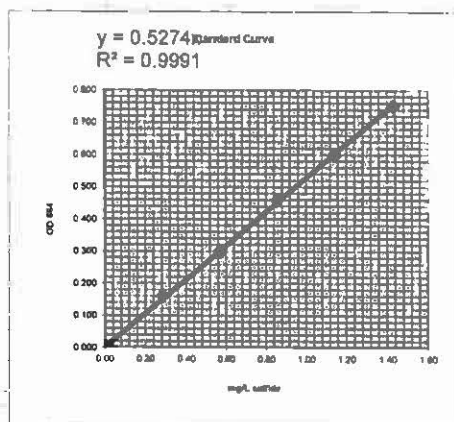
Dissolved Sulfide in Water: Computation Worksheet Methylene Blue Method (SOP #5550)

Standardization

	1	2	3
uL PAO titrant employed:	112	112	108
Working Std. Conc. (mg/L):		1.42933	

Result

Sample description	Dilution factor	OD664	Sulfide (mg/L)
Blank	---	---	---
1.0 mL working sulfide std.	---	0.161	0.29
2.0 mL working sulfide std.	---	0.300	0.57
3.0 mL working sulfide std.	---	0.460	0.86
4.0 mL working sulfide std.	---	0.599	1.14
5.0 mL working sulfide std.	---	0.751	1.43
3.0 mL spike	---	0.451	0.86
3.0 mL spike dupl.	---	0.450	0.85



1	5230G	5	0.025	0.24
2	5231G	5	0.021	0.20
3	5245G	5	0.005	ND
4	5246G	5	0.010	ND
5	5247G	5	0.000	ND

Reporting limit (mg/L) = 0.10

Recovery (%) = 99.6

Precision (RPD) = 0.22

Sample volume (ml): 1.00

Dilution factor 5

Sample Set Description:

Test No.:

Test Day:

Species:

Proj. No.: P865

X Bulk sediment porewaters

Test beaker porewaters

Overlying water

Analyst:

Date analysed:

RSC/JB

3/24/2015

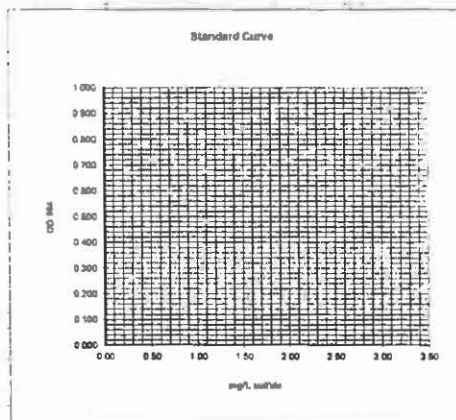
Dissolved Sulfide in Water: Computation Worksheet **Methylene Blue Method (SOP #5550)**

Standardization

	1	2	3
uL PAO titrant employed:	112	112	108
Working Std. Conc. (mg/L):		3.2	

Result

Sample description	Dilution factor	OD ₆₆₄	Sulfide (mg/L)
Blank	----	----	----
1.0 mL working sulfide std.	----	0.161	0.64
2.0 mL working sulfide std.	----	0.300	1.28
3.0 mL working sulfide std.	----	0.460	1.92
4.0 mL working sulfide std.	----	0.599	2.56
5.0 mL working sulfide std.	----	0.751	3.20
3.0 mL spike	----	0.451	
3.0 mL spike dupl.	----	0.450	



1	5230G	5	0.025
2	5231G	5	0.021
3	5245G	5	0.005
4	5246G	5	0.010
5	5247G	5	0.000
6			
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34			
35			
36			

Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

Sample volume (ml): 1.00

Dilution factor 5

Sample Set Description:

Test No.:

Test Day:

Species:

Proj. No.: P865

X Bulk sediment porewaters

Test beaker porewaters

Overlying water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet Salicylate Method (SOP #5492)

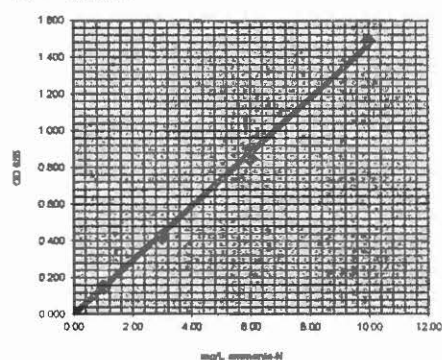
Result

Sample description		Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank		----	----	----		
1.0 mg/L NH ₃ -N Std.		----	0.149	1.00		
3.0 mg/L NH ₃ -N Std.		----	0.422	3.00		
6.0 mg/L NH ₃ -N Std.		----	0.851	6.00		
10.0 mg/L NH ₃ -N Std.		----	1.490	10.00		
3.0 mg/L spike		----	0.421	2.87		
3.0 mg/L spike dupl.		----	0.420	2.86		
5.0 mg/L 2nd source			0.710	4.84		
1	6	5	0.040	1.36	6.9	29.5
2	7	5	0.017	0.58	7.5	29.5
3	10	5	0.018	0.61	7.7	29.0
4	26	5	0.090	3.07	7.9	29.0
5	27	5	0.031	1.06	7.7	29.5
6	32	5	0.022	0.75	7.6	29.5
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$$y = 0.1467x$$

$$R^2 = 0.9983$$

Standard Curve



Reporting limit (mg/L) = 0.50

Recovery (%) = 95.5

Precision (RPD) = 0.24

2nd source (%) = 96.7

Sample volume (ml): 0.10

Dilution factor 5

Sample Set Description:

Test No.: 865-1

Test Day: 10

Species: *Eohaustorius***Sample Type (check)**

Bulk Sediment Porewaters

X Test Beaker Porewaters

Overlying Water

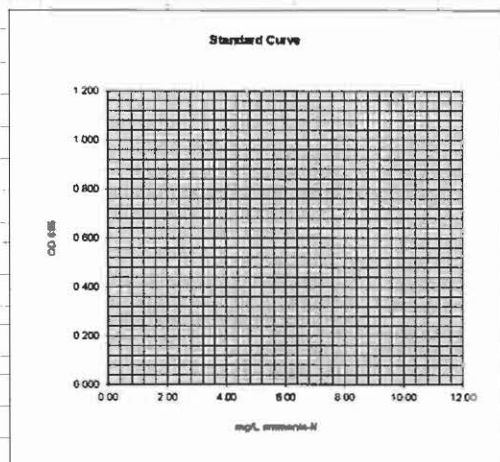
Analyst: JB

Date analyzed: 4/10/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet Salicylate Method (SOP #5492)

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	---	---	---		
1.0 mg/L NH ₃ -N Std.	---	0.149	1.00		
3.0 mg/L NH ₃ -N Std.	---	0.422	3.00		
6.0 mg/L NH ₃ -N Std.	---	0.851	6.00		
10.0 mg/L NH ₃ -N Std.	---	1.470	10.00		
3.0 mg/L spike	---	0.421			
3.0 mg/L spike dupl.	---	0.420			
5.0 mg/L 2nd source		0.710			
1 6	5	0.040		6.9	29.5
2 7	5	0.017		7.5	29.5
3 10	5	0.018		7.7	29.0
4 26	5	0.090		7.9	29.0
5 27	5	0.031		7.7	29.5
6 32	5	0.022		7.6	29.5



Reporting limit (mg/L) = 0.50

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.10

Dilution factor 5

Sample Set Description:

Test No.: 865-1

Test Day: 10

Species: *Eohaustorius***Sample Type (check)**

Bulk Sediment Porewaters

X Test Beaker Porewaters

Overlying Water

Analyst: JB

Date analyzed: 4/10/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet Salicylate Method (SOP #5492)

Result

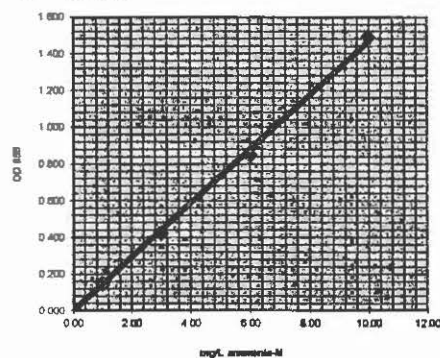
Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	---	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.149	1.00		
3.0 mg/L NH ₃ -N Std.	---	0.422	3.00		
6.0 mg/L NH ₃ -N Std.	---	0.851	6.00		
10.0 mg/L NH ₃ -N Std.	---	1.490	10.00		
3.0 mg/L spike	---	0.421	2.87		
3.0 mg/L spike dupl.	---	0.420	2.86		
5.0 mg/L 2nd source		0.710	4.84		

1	6	1	0.099	0.67
2	7	1	0.008	ND
3	10	1	0.021	0.14
4	26	1	0.068	0.46
5	27	1	0.027	0.18
6	32	1	0.007	ND
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				

$$y = 0.1467x$$

$$R^2 = 0.9983$$

Standard Curve



Reporting limit (mg/L) = 0.10

Recovery (%) = 95.5

Precision (RPD) = 0.24

2nd source (%) = 96.7

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-1

Test Day: 0

Species: *Eohaustorius***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

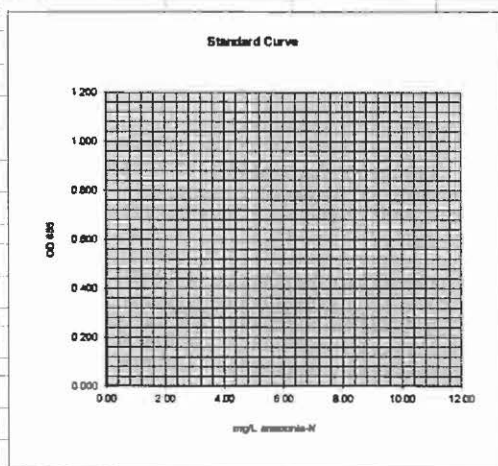
Analyst: JB

Date analysed: 4/10/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.149	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.422	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.851	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.490	10.00		
3.0 mg/L spike	----	0.421			
3.0 mg/L spike dupl.	----	0.420			
5.0 mg/L 2nd source		0.710			
1 6	1	0.079			
2 7	1	0.008			
3 10	1	0.021			
4 26	1	0.068			
5 27	1	0.027			
6 32	1	0.007			



Reporting limit (mg/L) =

0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-1

Test Day: 0

Species: *Eohaustorius***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst: JB

Date analysed: 4/10/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet Salicylate Method (SOP #5492)

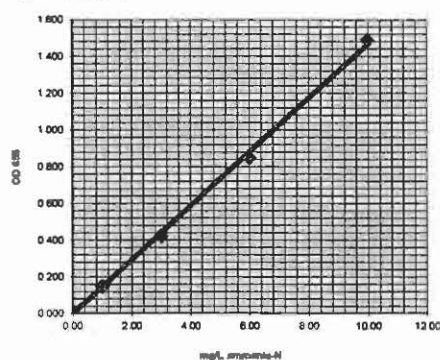
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.149	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.422	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.851	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.490	10.00		
3.0 mg/L spike	----	0.421	2.87		
3.0 mg/L spike dupl.	----	0.420	2.86		
5.0 mg/L 2nd source		0.710	4.84		
1 6	1	0.008	ND		
2 7	1	0.004	ND		
3 10	1	0.019	0.13		
4 26	1	0.169	1.15		
5 27	1	0.020	0.14		
6 32	1	0.000	ND		

$$y = 0.1467x$$

$$R^2 = 0.9983$$

Standard Curve



Reporting limit (mg/L) = 0.10

Recovery (%) = 95.5

Precision (RPD) = 0.24

2nd source (%) = 96.7

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-1

Test Day: 10

Species: *Eohaustorius***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst: JB

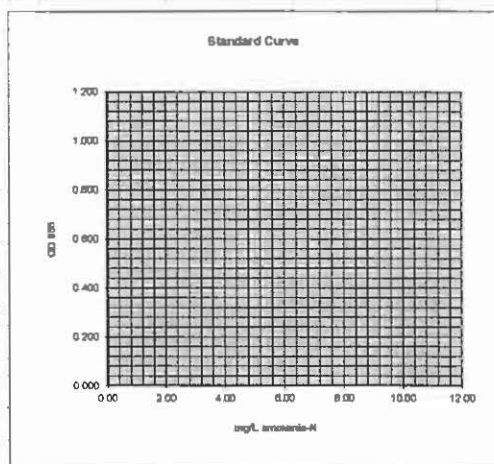
Date analysed: 4/10/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet Salicylate Method (SOP #5492)

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.149	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.422	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.857	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.490	10.00		
3.0 mg/L spike	----	0.421			
3.0 mg/L spike dupl.	----	0.420			
5.0 mg/L 2nd source		0.710			

1	6	1	0.008
2	7	1	0.004
3	10	1	0.019
4	26	1	0.169
5	27	1	0.020
6	32	1	0.000



Reporting limit (mg/L) =

0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-1

Test Day: 10

Species: *Eohaustorius***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst: JB

Date analysed: 4/10/2015

CHAIN-OF-CUSTODY RECORDS

CUSTODY TRANSFER

Printed: 03/19/15

ARI Job No: ZV36



4611 South 134th Place, Suite 100
Tukwila WA 98168
206-695-6200 206-695-6201 (fax)

ARI Project Manager: Cheronne Oreiro	Client Contact: Dan Berlin	Sampling Event: 110403-01.02	Samples Received: 02/05/15
	Client: Anchor QEA, LLC.	Project: West Waterway Sediment Char	Sample Site: NA

LOGNUM ARI ID	CLIENT ID	MATRIX	# CONTAINERS	ANALYTICAL REQUEST	ANALYTICAL REQUEST	ANALYTICAL REQUEST	COMMENTS
15-2108 ZV36D	WWD02-0-4-150205	Sediment	1	Bioassay			NAS # 52466
15-2109 ZV36E	WWD02-4-8-150205	Sediment	1	Bioassay			NAS # 52456

TEMP: 14.5 °C

PAGE 39 OF 47

Comments/Special Instructions 128326950355612155 128326950356908763 128326950356171571 128326950356999988	Relinquished By Printed Name Jennifer Millsap Company ARI Date/Time 3/19/15 1400	Received by (Signature) Printed Name GERALD IRISARRA Company NAS Date/Time 3-23-15 / 1325	Relinquished By Printed Name Company Date/Time	Received by (Signature) Printed Name Company Date/Time
---	--	--	---	--

CUSTODY TRANSFER

Printed: 03/19/15

ARI Job No: ZV66



4611 South 134th Place, Suite 100
Tukwila WA 98168
206-695-6200 206-695-6201 (fax)

ARI Project Manager: Cheronne Oreiro	Client Contact: Dan Berlin	Sampling Event: 110103-01.02	Samples Received: 02/06/15
	Client: Anchor QEA, LLC.	Project: West Waterway Sediment Char	Sample Site: NA

LOGNUM ARI ID	CLIENT ID	MATRIX	# CONTAINERS	ANALYTICAL REQUEST	ANALYTICAL REQUEST	ANALYTICAL REQUEST	COMMENTS
15-2221 ZV66A	WWD05-0-4-150206	Sediment	1	Bioassay			NAS # 5231G
15-2224 ZV66D	WWD12-0-4-150206	Sediment	1	Bioassay			NAS # 5247G

TEMP: 14.0 °C

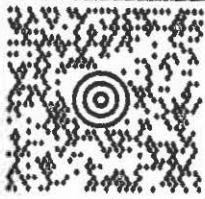
PAGE 40 OF 47

Comments/Special Instructions	Relinquished By <i>[Signature]</i>	Received by (Signature) <i>[Signature]</i>	Relinquished By	Received by (Signature)
	Printed Name Jennifer Millsap	Printed Name GERALD IRISARRI	Printed Name	Printed Name
	Company ARI	Company NAS	Company	Company
	Date/Time 3/19/15 1400	Date/Time 3-23-15 1315	Date/Time	Date/Time

SHIP
TO:

20/Mar/2015 03:10 9729

**NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639**



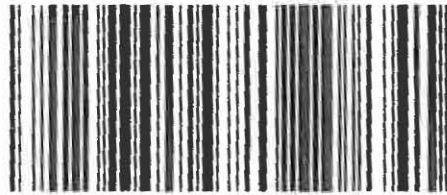
OR 973 2-01



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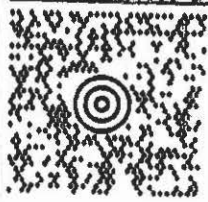


SHIPMENT LICENSE US 9729 MAR 20 03:09:12 2015 HIP 1.3.1 ZP4505

SHIP
TO:

20/Mar/2015 03:37 9729

**NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639**



OR 973 2-01



1Z8328950355612155

UPS GROUND

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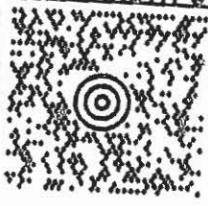


SHKSHW LICENSE US 9729 MAR 20 03:36:19 2015 HIP 14.3.1 204505

SHIP
TO:

20/Mar/2015 03:43 9729

**NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639**



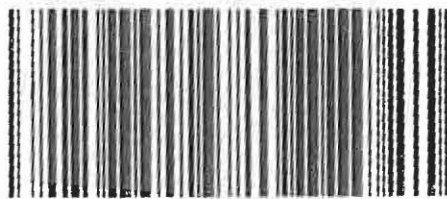
OR 973 2-01



128326950356999988

UPS GROUND

TRACKING #: 1Z 832 695 03 5699 9988



SHKSHUL LICENSE US 9729 MAR 20 03:42:54 2015 H1P 14.3.1 274500

SAMPLE RECEIVING
12067 894-6200
PMT LABS INC
1611 S 164TH PL
TUMULT WA 98148 2212

30 L.W

(01

SHIP TO:

SAMPLE RECEIVING

541) 267-7225

WESTERN AQUARIUM SCIENCES

1500 12th Bldg Bldg

100

97366 3639

TEMP: 15-5°C

MAS# 52316

373 2-01

CUSTODY SEAL

Date 3/19/15
Signature [Signature]

Thermo
SCIENTIFIC

00006

CUSTODY SEAL

Date 3/19/15

Signature [Signature]

Thermo
SCIENTIFIC

90009

CUSTC

Date 3/1

Signature [Signature]

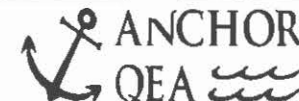
SEA L

[Signature]

DO NOT

Thermo
SCIENTIFIC

90006



Chain of Custody Information				Sediment and Field QC													Comments
Line	Field Sample ID	Collection Date/Time	Matrix	No. of Containers	Total Metals	SVOCs/PAHs/PCBs/pesticides	Bulk TBT	Dioxin/furans	Grain Size	Ammonia	TS/TOC/TVS	Total Sulfides	Archive (Chemistry)	Asbestos (Bioassay)			
1	WWD-REF-150319	3/19/15 1105	SE	2										X	NA# 52306		
2			SE														
3			SE														
4			SE														
5			SE														
6			SE														
7			SE														
8			SE												TEMP BLANK = 4.2°C		
9			SE														
10			SE														
11			SE														
12			SE														
13			SE														
14			SE														

1 See project SAP/QAPP for analyte lists and test methods

2 email sample confirmation report to labdata@anchorqea.com

Additional notes/comments:

NITROGEN IN HEADSPACE AS PRESERVATIVE

Relinquished By:	Company: Anchor QEA LLC.	Received By:	Company: ARI
<i>SARA Bitterman</i>	3/19/15 15:07	<i>Guy Buhle</i>	NAS 3-2075 1135
Signature/Printed Name	Date/Time	Signature/Printed Name	Date/Time

Relinquished By:	Company:	Received By:	Company:
Signature/Printed Name	Date/Time	Signature/Printed Name	Date/Time

FedEx Express **US Airbill**

8656 3169 3053

0200

Form 10 No.

FedEx Retrieval Copy

1 From

Date 11/11/01 Sender's FedEx Account Number 2155 11224

Sender's Name David L. Linn Phone 206 221 1111

Company David L. Linn

Address 220 Olive Way, Suite 140 Dept./Floor/Suite/Room

City Seattle State WA ZIP 98101

2 Your Internal Billing Reference www

3 To

Recipient's Name David L. Linn Phone 206 221 1111

Company Northwestern Pacific Science

Recipient's Address 220 Olive Way, Suite 140 Dept./Floor/Suite/Room

We cannot deliver to P.O. boxes or P.O. ZIP codes

Address 220 Olive Way, Suite 140

City Seattle State WA ZIP 98101

4a Express Package Service

☒ **FedEx Priority Overnight** Next business morning * Friday shipments will be delivered on Monday, unless SATURDAY Delivery is selected

☐ **FedEx Standard Overnight** Next business afternoon * Saturday Delivery NOT available

☐ **FedEx 2Day** Second business day * Thursday shipments will be delivered on Monday, unless SATURDAY Delivery is selected

☐ **FedEx Express Saver** Third business day * Saturday Delivery NOT available

☐ **FedEx 1Day Freight*** Next business day ** Friday shipments will be delivered on Monday, unless SATURDAY Delivery is selected

☐ **FedEx 2Day Freight** Second business day ** Thursday shipments will be delivered on Monday, unless SATURDAY Delivery is selected

☐ **FedEx 3Day Freight** Third business day ** Saturday Delivery NOT available

* To meet local times

5 Packaging

☒ **FedEx Envelope***

☐ **FedEx Pak*** Includes FedEx Small Pak, FedEx Large Pak, and FedEx Sturdy Pak

☐ **FedEx Box**

☐ **FedEx Tube**

☒ **Other**

* Declared value limit \$500

6 Special Handling

☒ **SATURDAY Delivery** Not available for FedEx Standard Overnight, FedEx First Overnight, FedEx Express Saver, or FedEx 2Day Freight

☐ **HOLD Weekday at FedEx Location** Not available for FedEx First Overnight

☐ **HOLD Saturday at FedEx Location** Available ONLY for FedEx Priority Overnight and FedEx 2Day to select locations

Does this shipment contain dangerous goods?

☒ **No**

☐ **Yes** One box must be checked. As per attached Shipper's Declaration

☐ **Yes** Shipper's Declaration not required

☐ **Dry Ice** Dry Ice, 8 UN 1845

☐ **Cargo Aircraft Only**

Dangerous goods (including dry ice) cannot be shipped in FedEx packaging

7 Payment Bill to: Enter FedEx Acct No. or Credit Card No. below

☒ **Sender** Acct No. 2155 11224 Sector 1 will be billed

☐ **Recipient**

☐ **Third Party**

☐ **Credit Card**

☐ **Cash/Check** Obtain Recip Acct No.

Total Packages 1 Total Weight 3.65

Our liability is limited to \$100 unless you declare a higher value. See the current FedEx Service Guide for details. Credit Card Auth.

8 Residential Delivery Signature Options If you require a signature, check Direct or Indirect:

☐ **No Signature Required** Package may be left without obtaining a signature for delivery

☐ **Direct Signature** Someone at recipient's address may sign for delivery. Fee applies.

☐ **Indirect Signature** If no one is available at recipient's address, someone at a neighboring address may sign for delivery. Fee applies.

520

Rev. Date 10/08/01 Part 1/130231-01/094-2208 FedEx-PRINTED IN U.S.A. 877



8656 3169 3053

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

Test No. 999-3406 Client: QC Test Investigator REVIEWED
PAGES 1-7
-652
 Test Type (ranging/definitive) definitive Test Length (hr) 96
 Species Eohaustorius estuarius

STUDY MANAGEMENT

Client: QC Test
 Client's Study Monitor: N/A
 Testing Laboratory: Northwestern Aquatic Sciences
 Test Location: Newport Laboratory
 Laboratory's Study Personnel:
 Proj. Man./Study Dir. G.J. IRISSARI 651
 QA Officer L.K. Nemeth
 1. Yves Nakagawa 2. GABRIEL
 3. Lowen Brady 4. LS
 5. LS 6. LS

Study Schedule:
 Test Beginning: 3-31-15 1020 Test Ending: 4-4-15 1030

TEST MATERIAL

Description: MALLINCKRODT (NO LOT #)
Ammonia prepared from ammonium chloride; stock prepared 12/31/13,
10,000 mg/L (=10.0 mg/mL) expressed as N

DILUTION WATER

Description: Yaquina Bay, Oregon, sea water
 Date of Preparation/Collection: 3-25-15
 Water Quality: Cond.(umhos/cm): — Salinity (ppt) 25.0 pH 8.1
 Hardness (mg/L as CaCO₃): — Alkalinity (mg/L as CaCO₃): —
 Treatments: Filtered to ≤ 0.45 um, salinity adjusted with Milli-Q deionized water, aerated

TEST LOCATION

Test conducted in (circle one): room 1 room 2 trailer water bath other: ROOM #4

Randomization chart:

NORTHWESTERN AQUATIC SCIENCES
ACUTE TOXICITY TEST (ALL SPECIES)

PROTOCOL NO. NAS-XXX-RA1

Test No. 999-3406 Client: QC Test Investigator

TEST ORGANISMS

Species: Eohaustorius estuarius Age: adult Size:
Source: Yaquina Bay, Oregon

If field-collected:

Date Collected: 3-26-15
Field conditions: Interstitial temperature: 12.0°C Interstitial salinity: 14.0 ppt

Acclimation Data:

Date	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Feeding		Water changes	Comments
					amount	description		
3-26-15	15.6	7.7	20.0	8.6	NOT	FED	YES	
3-27-15	15.5	8.0	20.0	8.3	"	"	YES	
3-28-15	15.6	8.1	26.5	8.1	"	"	-	
3-29-15	15.5	8.1	26.5	8.2	"	"	YES	
3-30-15	15.6	8.1	28.0	8.2	"	"	-	
3-31-15	15.7	8.1	27.0	8.2	"	"	-	
Mean	15.6	8.1	24.7	8.3				
S.D.	0.1	0.2	3.7	0.2				
(N)	6	6	6	6				

Photoperiod during acclimation: Constant light

TEST PROCEDURES AND CONDITIONS

Test concentrations (50% series recommended): 500, 250, 125, 62, 31, and 0 mg/L

Test chamber: 250 mL beakers Test volume: 100 mL

Replicates/treatment: 2 Organisms/treatment: 20 (10/repl)

Test water changes: None Aeration during test: None

Feeding: None

Duration: 24-hr, 48-hr, 96-hr Test temperature (deg.C): 15.0 +/- 1.0

Beaker placement: Stratified randomization Photoperiod: Constant light

Test salinity (ppt): 28.0 +/- 1.0

MISCELLANEOUS NOTES

Test Solution Preparation:

Test Conc. (mg/L)	mL of stock (10.0 mg/mL) per 200 mL	
500	10.0	Bring up to 200 mL with dilution water, then split between 2 replicates
250	5.0	
125	2.5	
62	1.3	
31	0.65	
0	0	

3-31-15
621

NORTHWESTERN AQUATIC SCIENCES
ACUTE TOXICITY TEST (ALL SPECIES)

PROTOCOL NO. NAS-XXX-RA1

Test No. 999-3406 Client QC Test Investigator

DAILY RECORD SHEET

Day 0 (3/31/15) LS

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors	
					A	B
1. 500	15.6	7.5	29.0	8.0	10	10
2. 250	15.8	7.6	29.0	8.1	10	10
3. 125	15.7	7.8	28.0	8.0	10	10
4. 62	15.7	7.9	28.0	8.0	10	10
5. 31	15.7	8.0	28.0	8.0	10	10
6. 0	15.9	8.1	28.0	8.1	10	10

Day 1 (4/1/15) LS/632

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors	
					A	B
1. 500	15.1	7.6	28.5	8.0	5(5D)	4(4D)(6D)
2. 250	15.1	7.7	28.0	8.1	8(2D)	10
3. 125	15.1	7.8	28.0	7.9	10	10
4. 62	15.1	7.9	28.0	7.9	10	10
5. 31	15.1	7.9	27.5	8.0	10	10
6. 0	15.1	8.0	27.5	8.0	10	10

Day 2 (4/2/15) LS/632

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors	
					A	B
1. 500	15.1	7.6	29.0	7.3	0(5D)	0(4D)
2. 250	15.2	7.8	29.0	7.5	4(4D)	7(3D)
3. 125	15.1	7.8	29.0	7.7	10	10
4. 62	15.2	7.9	29.0	7.7	10	10
5. 31	15.2	7.9	29.0	7.7	10	10
6. 0	15.2	8.0	28.0	7.7	10	10

Day 3 (4/3/15) LS/632

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors	
					A	B
1. 500	—	—	—	—	0	0
2. 250	15.0	7.7	29.0	7.6	4	6(10)
3. 125	15.0	7.8	29.0	7.4	10	10
4. 62	15.0	7.9	29.5	7.3	10	10
5. 31	15.0	7.9	29.0	7.2	10	10
6. 0	15.0	8.0	29.0	7.5	10	10

Day 4 (4/4/15) LS

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors	
					A	B
1. 500	—	—	—	—	0	0
2. 250	14.8	7.7	29.0	7.7	2(2D)	5(10)
3. 125	14.8	7.8	29.5	7.4	10	10
4. 62	14.9	7.9	29.5	7.2	10	10
5. 31	14.9	8.0	29.0	7.4	10	10
6. 0	14.8	8.1	29.5	7.6	10	10

WQ: Mean
SD
n

(SEE PAGE 4)

Water Quality Data - test #999-3406 Eoh QC-NH4 test						
Day	Concentration	Temperature	pH	Salinity	DO	
0	500	15.6	7.5	29.0	8.0	
0	250	15.8	7.6	29.0	8.1	
0	125	15.7	7.8	28.0	8.0	
0	62	15.7	7.9	28.0	8.0	
0	31	15.7	8.0	28.0	8.0	
0	0	15.9	7.1	28.0	8.1	
1	500	15.1	7.6	28.5	8.0	
1	250	15.1	7.7	28.0	8.1	
1	125	15.1	7.8	28.0	7.9	
1	62	15.1	7.9	28.0	7.9	
1	31	15.1	7.9	27.5	8.0	
1	0	15.1	8.0	27.5	8.0	
2	500	15.1	7.6	29.0	7.3	
2	250	15.2	7.8	29.0	7.5	
2	125	15.1	7.8	29.0	7.7	
2	62	15.2	7.9	29.0	7.7	
2	31	15.2	7.9	29.0	7.7	
2	0	15.2	8.0	28.0	7.7	
3	500					
3	250	15.0	7.7	29.0	7.6	
3	125	15.0	7.8	29.0	7.4	
3	62	15.0	7.9	29.5	7.3	
3	31	15.0	7.9	29.0	7.2	
3	0	15.0	8.0	29.0	7.5	
4	500					
4	250	14.8	7.7	29.0	7.7	
4	125	14.8	7.8	29.5	7.4	
4	62	14.9	7.9	29.5	7.2	
4	31	14.9	8.0	29.0	7.4	
4	0	14.8	8.1	29.5	7.6	
MEAN		15.2	7.8	28.7	7.7	
SD		0.3	0.2	0.6	0.3	
N		28	28	28	28	
MIN		14.8	7.1	27.5	7.2	
MAX		15.9	8.1	29.5	8.1	

data entry verified against laboratory bench sheets

5-14-15
JZC

CETIS Summary Report

Report Date: 21 Apr-15 12:02 (p 1 of 1)
 Test Code: 999-3406 15-4015-9529

Reference Toxicant 96-h Acute Survival Test						Northwestern Aquatic Sciences					
Batch ID:	17-5314-8040	Test Type:	Survival	Analyst:							
Start Date:	31 Mar-15 10:20	Protocol:		Diluent:	Yaquina Bay Seawater						
Ending Date:	04 Apr-15 10:30	Species:	Eohaustorius estuarius	Brine:							
Duration:	4d 0h	Source:	Field Collected	Age:							
Sample ID:	13-7684-7649	Code:	52110721	Client:	Internal Lab						
Sample Date:	31 Mar-15 10:20	Material:	Ammonia as nitrogen	Project:							
Receive Date:	31 Mar-15 10:20	Source:	Reference Toxicant								
Sample Age:	NA	Station:									
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
06-8710-0927	Proportion Survived	125	250	176.8	18.6%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	mg/L	95% LCL	95% UCL	TU	Method				
01-5088-3539	Proportion Survived	EC50	225.3	194.3	261.2		Spearman-Kärber				
Proportion Survived Summary											
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	2	1	1	1	1	1	0	0	0.0%	0.0%
31		2	1	1	1	1	1	0	0	0.0%	0.0%
62		2	1	1	1	1	1	0	0	0.0%	0.0%
125		2	1	1	1	1	1	0	0	0.0%	0.0%
250		2	0.35	0	1	0.2	0.5	0.15	0.2121	60.61%	65.0%
500		2	0	0	0	0	0	0	0		100.0%
Proportion Survived Detail											
C-mg/L	Control Type	Rep 1	Rep 2								
0	Dilution Water	1	1								
31		1	1								
62		1	1								
125		1	1								
250		0.2	0.5								
500		0	0								

CETIS Test Data Worksheet

Report Date: 21 Apr-15 12:01 (p 1 of 1)
 Test Code: 15-4015-9529/999-3406

Reference Toxicant 96-h Acute Survival Test						Northwestern Aquatic Sciences
Start Date:	31 Mar-15 10:20	Species:	Eohaustorius estuarius	Sample Code:	52110721	
End Date:	04 Apr-15 10:30	Protocol:		Sample Source:	Reference Toxicant	
Sample Date:	31 Mar-15 10:20	Material:	Ammonia as nitrogen	Sample Station:		
C-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	8	10	10	
0	D	2	9	10	10	
31		1	2	10	10	
31		2	7	10	10	
62		1	4	10	10	
62		2	1	10	10	
125		1	5	10	10	
125		2	3	10	10	
250		1	12	10	2	
250		2	11	10	5	
500		1	6	10	0	
500		2	10	10	0	

data entry verified against laboratory bench sheets 5-13-15 jmf

Amphipod, Eohaustorius estuarius, acute reference toxicant test

Northwestern Aquatic Sciences

Test Type: Survival

Organism: Eohaustorius estuarius (Amphipod)

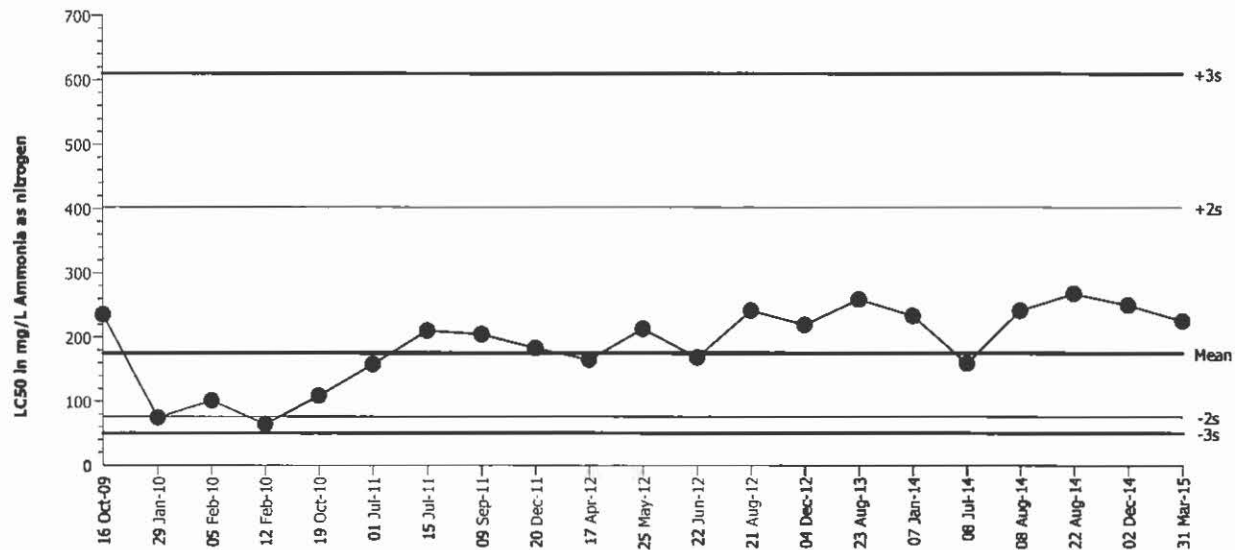
Material: Ammonia as nitrogen

Protocol: All Protocols

Endpoint: Proportion Survived

Source: Reference Toxicant-REF

Amphipod, Eohaustorius estuarius, acute reference toxicant test



Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2009	Oct	16	10:40	235.1	60.07	0.7095			04-6966-4431	05-3952-8779
2	2010	Jan	29	9:50	74.3	-100.7	-2.06	(-)		03-5377-7187	20-4403-3162
3		Feb	5	10:05	101.2	-73.83	-1.317			08-1570-8058	20-8812-4092
4			12	10:50	63.96	-111.1	-2.421	(-)		01-1330-5289	09-8715-3590
5		Oct	19	11:15	108.5	-66.57	-1.151			09-2966-0485	20-5470-9414
6	2011	Jul	1	14:15	157.4	-17.65	-0.2556			06-2971-2336	11-3536-4545
7			15	14:30	210.1	35.06	0.439			17-7989-3327	04-8659-6502
8		Sep	9	13:35	204.4	29.38	0.3731			05-7587-2108	04-3384-8763
9		Dec	20	10:10	182.9	7.863	0.1057			10-7131-5711	10-4572-4810
10	2012	Apr	17	10:05	164.9	-10.17	-0.1439			12-4744-0558	10-6629-0235
11		May	25	9:40	212.9	37.91	0.4714			15-2875-0348	10-8612-8592
12		Jun	22	9:50	168.4	-6.664	-0.09333			01-7310-7337	20-4287-9582
13		Aug	21	11:10	241.4	66.4	0.7733			02-9790-0210	21-1650-5427
14		Dec	4	9:40	218.8	43.81	0.5371			09-9576-8200	14-2558-1248
15	2013	Aug	23	9:50	258.8	83.78	0.9404			20-0854-2243	05-9018-4964
16	2014	Jan	7	10:55	233.3	58.22	0.6904			11-7316-9607	20-3106-7745
17		Jul	8	11:25	159.2	-15.81	-0.2277			14-3717-0154	16-1977-5683
18		Aug	8	11:50	241.4	66.35	0.7728			16-1309-9337	20-0447-5176
19			22	9:45	267.9	92.85	1.023			15-9998-3863	14-0511-5604
20		Dec	2	9:45	249.9	74.91	0.8566			08-8221-8356	03-3293-3808
21	2015	Mar	31	10:20	225.3	50.28	0.6071			15-4015-9529	01-5088-3539

SECTION C

Neanthes arenaceodentata sediment bioassay 865-2 data report

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 865-2

Title: Juvenile *Neanthes* 20-day sediment toxicity test of estuarine sediments as part of Port of Seattle – West Waterway Deepening project.

Protocol: NAS-XXX-NA4, June 20, 1990. Rev. 4 (3-1-05). Based on: Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995), with modifications as specified by the Dredged Material Management Program (DMMP, formerly Puget Sound Dredged Disposal Analysis Program or PSDDA). Dredged Material Evaluation and Disposal Procedures User Manual, December 2014.

STUDY MANAGEMENT

Study Sponsor: Anchor QEA, 720 Olive Way, Suite 1900, Seattle, Washington 98101.

Sponsor's Study Monitor: Ms. Cindy Fields

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, Oregon 97365.

Test Location: Newport Laboratory.

Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Mngr./ Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, Ph.D., Sr. Aq. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; J. B. Brown, B.S., D.V.M., Assoc. Aq. Toxicol.; Y. Nakahama, Sr. Tech.; L. Brady, Tech.

Study Schedule:

Test Beginning: 3-31-15, 1040 hrs.

Test Ending: 4-20-15, 1130 hrs.

Disposition of Study Records: All raw data, reports, and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

TEST MATERIAL

Control Sediment: Control sediment (NAS Sample #5250G) was collected from the *Eohaustorius estuarius* amphipod collection site in lower Yaquina Bay, Oregon, on 3-25-15. The sediment was sieved through a 0.5-mm stainless steel screen and stored at 4°C in the dark.

Test Sediments: Four test sediments and one reference sediments were tested. Details follow:

NAS Sample No.	5231G	5245G	5246G
Sample Description	WWD05-0-4-150206	WWD02-4-8-150205	WWD02-0-4-150205
Collection Date	2-6-15	2-5-15	2-5-15
Receipt Date	3-23-15	3-23-15	3-23-15
Interstitial Salinity (‰)	29.0	29.5	29.5
NAS Sample No.	5247G	5230G	
Sample Description	WWD12-0-4-150206	WWD-REF-150319	
Collection Date	2-6-15	3-19-15	
Receipt Date	3-23-15	3-20-15	
Interstitial Salinity (‰)	29.0	27.0	

Storage: Samples were stored at 4°C in the dark.

Treatments: The samples were minimally homogenized by mixing with stainless steel implements.

TEST WATER

Source: Yaquina Bay, Oregon

Date of Collection: 3-25-15

Water Quality: Salinity 28.0‰, pH 8.0

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, salinity-adjusted with MilliQ® deionized water, aerated.

TEST ORGANISMS

Species: *Neanthes arenaceodentata*, marine polychaete worm

Age: 2-3 week post-emergence juveniles

Initial wt.: 0.48 mg

Source: Laboratory cultures at Aquatic Toxicology Support, Bremerton, Washington. Worms were received on 3-27-15.

Acclimation: Average conditions during the five days prior to testing were: temperature, $19.9 \pm 1.5^\circ\text{C}$; pH, 7.8 ± 0.3 ; salinity, 28.0 ± 0.7 ‰; dissolved oxygen, 7.3 ± 0.1 mg/L.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 1 L covered borosilicate glass beakers.

Test Volumes: 175 ml of test, reference, or control sediment; 950 ml total volume.

Replicates/Treatment: 5 (plus one water-quality replicate)

Sediment Salinity Adjustment: After sediment and overlying water were added to beakers on test day -1, sediments with interstitial salinities below 20.0 ppt were stirred with the overlying water and allowed to settle overnight with aeration. No sediments in this test required salinity adjustment.

Organisms/Treatment: 25 (5/replicate)

Water Volume Changes: One third of the seawater in each beaker was replaced every third day.

Aeration: Provided using a 1-ml glass pipette with the tip 3-4 cm below the water surface. Air was bubbled at a low rate (150-300 ml/min) so as not to disturb the sediment surface.

Feeding: Animals were fed 40 mg TetraMarine® per beaker every other day.

Acceptance Criteria: Results are valid if mean control survival is at least 90%. DMMP and SMS require control sediment mortality of $\leq 10\%$ and a growth rate of > 0.38 mg/individual/day.

Performance Criteria: For DMMP and SMS testing, the reference sediment mortality should be $\leq 20\%$ with a growth rate of $\geq 80\%$ that of the negative control sediment.

Effects Criteria: 1) survival after 20 days, 2) average individual biomass, 3) average individual growth rate and 4) average individual growth rate as ash-free dry weight. Death is defined as no visible appendage movement or response to tactile stimulation. Missing worms are considered dead.

Water Quality and Other Test Conditions: The temperature, dissolved oxygen, salinity, and pH were measured in the overlying water of one replicate water quality beaker on test days 0 and 20, and on test days 3, 6, 9, 12, 15, and 18 prior to test solution renewal. Total dissolved sulfide and total ammonia-N were measured in the overlying water of the water quality replicate test chamber on days 0 and 20. Total ammonia-N was also measured in the overlying water of the water quality replicate test chamber on day 3 prior to water renewal. Interstitial total ammonia-N and total dissolved sulfide were measured in bulk sediments. Interstitial water samples were obtained by centrifugation or by settling. Total soluble sulfide and total ammonia-N were measured using Hach reagents based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. Un-ionized ammonia-N was computed using "Un-ionized Ammonia Calculator", v1.0 (Dr. Landon Ross, Florida Department of Environmental Protection). The photoperiod was constant light.

DATA ANALYSIS METHODS

Percent survival, individual biomass, and individual growth rate at the end of the test were determined from the final observations according to the formulas:

Percent Mortality = $100 \times ([\text{initial no. of worms} - \text{no. of surviving worms}] / \text{initial no. of worms})$

Individual biomass = total dry wt. of worms/number of surviving worms weighed

Individual growth rate = (individual biomass - the initial dry wt.)/20 test days

Individual ash-free biomass = total ash-free wt. of worms/number of surviving worms weighed

Individual ash-free growth rate = (individual ash-free biomass - initial ash-free weight)/20 test days

Control and treatment means and standard deviations for the biological endpoints described above and for water quality data were computed using Microsoft EXCEL 2010. The software used for statistical comparisons was BioStat (version Feb 9, 2006 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. Mean individual growth rate in each test sediment was compared against that in the appropriate reference sediment. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.05 level of significance.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The reference toxicant test is a standard multi-concentration toxicity test using ammonia expressed as $\text{NH}_3\text{-N}$ and administered as NH_3Cl , to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix II.

Test No.: 999-3407

Reference Toxicant and Source: Ammonia as ammonium chloride, Mallinckrodt, 10.0 mg/mL ammonia-as-N stock prepared 12-31-13

Test Date: 3-31-15

Dilution Water Used: Yaquina Bay, Oregon, seawater; 28.0%

Result: The 96-hr LC_{50} was 242 mg/L $\text{NH}_3\text{-N}$. This result is within the laboratory's control chart warning limits (186–314 mg/L $\text{NH}_3\text{-N}$).

RESULTS AND DISCUSSION

Observations of water quality parameters during the test are summarized in Table 1. Individual water quality measurements are located in the raw data (Appendix II).

Except as noted above, all measurements of standard water quality parameters were within protocol specified ranges (Table 1). Sulfides were not detected in the overlying bioassay water (detection limit 0.02 mg/L). Total ammonia-N ranged from <0.1 mg/L to 2.5 mg/L (maximum un-ionized ammonia 0.095 mg/L).

Interstitial total ammonia-N concentrations in the bulk sediment ranged from 1.0 to 11.7 mg/L (maximum 0.553 mg/L un-ionized ammonia), with sulfide concentrations measuring <0.1 to 0.2 mg/L. See Table 2.

Table 3 shows the effects of test sediment exposures on survival and growth of *Neanthes*. The test met the acceptability criterion ($\geq 90\%$) for control survival; mean survival in the control was 100%. The individual growth rate (dry wt) in the controls averaged 0.58 mg/day/worm. This meets the DMMP recommendation for a minimum growth rate of 0.38 mg/day/worm for *Neanthes*. The average initial weight of worms was 0.48 mg, which was slightly lower than the recommended range of 0.5 - 1.0 mg. The reference sediments included in the study met the performance standard requirement that mortality in the reference sediment should be $\leq 20\%$; mortality in WWD-REF-150319 was 8.0%. The growth rate criterion was met for the reference sediment. According to DMMP criteria, the mean individual growth rate in the reference sediment should be $\geq 80\%$ of the mean individual growth rate in the control sediment. Control growth rate was 0.58 mg/day/worm. The growth rate in reference sediment WWD-REF-150319 was 0.65 mg/day/worm; corresponding to 112% of the control growth rate. Reference sediments also met the acceptability criterion in terms of ash-free growth rate. The control ash-free dry weight was 0.44 mg/day/worm, and reference sediment WWD-REF-150319 was 0.49 mg/day/worm, representing 111% of the control growth rate.

The test control acceptance and reference sediment performance criteria for survival were met, as was the growth criterion for the control sediment. Positive control performance was within the laboratory's acceptance

limits. The growth criterion for the reference sediment was met. It is concluded, therefore, that the test has developed acceptable data for use in making management decisions.

Test sediment WWD12-0-4-150206 was compared to the control sediment due to grain size considerations. All other test sediments were compared to the reference WWD-REF-150319. None of the test sediments exhibited statistically significantly ($\alpha = 0.05$) decreased individual growth rate and ash-free growth rate compared with reference sediment or negative control (Table 3).

Interpretation was based on guidelines from the Dredged Material Evaluation and Disposal Procedures User Manual, December 2014. If the mean individual growth rate of a test sediment is <80% of the mean negative control growth rate, and <70% (dispersive) or <50% (nondispersive) of the mean reference sediment growth rate, and statistically different ($\alpha = 0.05$) from the reference sediment, it fails under the single-hit rule. None of the test sediments failed the single-hit criteria for dispersive or non-dispersive rules under these guidelines (Table 4).

STUDY APPROVAL

Donald Scissari 6/3/15
Project Manager/ Study Director Date

Julie R. Fiore 6-3-15
Quality Assurance Unit Date

Linda K. Hemmick 6/2/15
Assistant Laboratory Director Date

Table 1. Summary of overlying water quality conditions during tests of the polychaete, *Neanthes arenaceodentata*, exposed to marine sediments.

Parameter	Mean \pm SD	Minimum	Maximum	N
Temperature ($^{\circ}$ C)	20.0 \pm 0.2	19.5	20.4	48
pH	8.0 \pm 0.1	7.8	8.6	48
Salinity (‰)	28.9 \pm 0.8	28.0	30.0	48
Dissolved Oxygen (mg/L)	6.8 \pm 0.3	6.1	7.3	48
Dissolved Sulfide (mg/L)	---	<0.02	<0.02	12
Total Ammonia-N (mg/L)	---	<0.1	2.5	18
Un-ionized Ammonia (mg/L)	---	0.005	0.095	18

Table 2. Summary of interstitial water quality conditions during tests of the polychaete, *Neanthes arenaceodentata*, exposed to marine sediments.

Parameter	Mean \pm SD	Minimum	Maximum	N
Salinity (‰)	28.8 \pm 1.0	27.0	29.5	5
pH	7.9 \pm 0.2	7.5	8.1	5
Dissolved Sulfide (mg/L)	---	<0.1	0.2	5
Total Ammonia-N (mg/L)	---	1.0	11.7	5
Un-ionized Ammonia (mg/L)	---	0.039	0.553	5

Table 3. Means and standard deviations (n=5) of percent mortality; individual biomass and ash-free biomass; and individual growth rate and ash-free growth rate of *Neanthes arenaceodentata* exposed for 20 days to marine sediments.

Sample description	Percent mortality (20-days)	Individual biomass (mg)	Individual ash-free biomass (mg)	Individual growth rate (mg/day/worm)	Individual ash-free growth rate (mg/day/worm)
Control (NAS# 5250G)	0.0 \pm 0.0	12.1 \pm 2.7	9.2 \pm 1.9	0.58 \pm 0.14	0.44 \pm 0.10
WWD02-0-4-150205 (NAS# 5246G)	12.0 \pm 17.9	15.6 \pm 2.0	12.3 \pm 1.2	0.75 \pm 0.10	0.59 \pm 0.06
WWD02-4-8-150205 (NAS# 5245G)	4.0 \pm 8.9	15.0 \pm 4.3	11.7 \pm 4.3	0.73 \pm 0.22	0.57 \pm 0.22
WWD05-0-4-150206 (NAS# 5231G)	8.0 \pm 11.0	14.3 \pm 1.7	11.3 \pm 1.3	0.69 \pm 0.09	0.55 \pm 0.06
WWD12-0-4-150206 (NAS# 5247G) ¹	0.0 \pm 0.0	14.8 \pm 2.0	11.2 \pm 1.5	0.72 \pm 0.10	0.54 \pm 0.07
WWD-REF-150319 (NAS# 5230G)	8.0 \pm 11.0	13.4 \pm 2.2	10.3 \pm 1.5	0.65 \pm 0.11	0.49 \pm 0.07

¹ Compared to the control sediment per client request.

*Growth rate was significantly lower than that in reference sediment (p<0.05).

‡Growth rate was significantly lower than that in control sediment (p<0.05).

Table 4. Single-hit criteria interpretation of *Neanthes* juvenile infaunal growth test data.

Sample description	Individual ash-free growth rate (mg/day)	Significantly different from the reference at $\alpha = 0.05$?	Percent of reference value	Percent of negative control	Failure under 1-hit dispersive rule? (T/R <70%)	Failure under 1-hit nondispersive rule? (T/R <50%)
Control (NAS# 5250G)	0.44 ± 0.10	---	---	---	---	---
WWD02-0-4-150205 (NAS# 5246G)	0.59 ± 0.06	No	120	134	No	No
WWD02-4-8-150205 (NAS# 5245G)	0.57 ± 0.22	No	116	130	No	No
WWD05-0-4-150206 (NAS# 5231G)	0.55 ± 0.06	No	112	125	No	No
WWD12-0-4-150206 (NAS# 5247G) ¹	0.54 ± 0.07	No ¹	110	123	No	No
WWD-REF-150319 (NAS# 5230G)	0.49 ± 0.07	---	---	---	---	---
If the mean individual growth rate of a test sediment is <80% of the mean negative control growth rate, and <70% (dispersive) or <50% (nondispersive) of the mean reference sediment growth rate, and statistically different ($\alpha = 0.05$) from the reference sediment, it fails under the single-hit rule.						
¹ Compared to the control sediment.						

APPENDIX I
PROTOCOL

TEST PROTOCOL

JUVENILE POLYCHAETE, *NEANTHES ARENACEODENTATA*, 20-DAY SOLID PHASE SEDIMENT BIOASSAY

1. INTRODUCTION

- 1.1 Purpose of Study: The purpose of this study is to identify marine sediments that are toxic to a marine polychaete worm. .
- 1.2 Summary of Method: The 20-day static renewal test is performed using laboratory-cultured, 2-3 week post-emergence juvenile worms purchased from a supplier. Test sediments are placed in the bottom of 1-liter glass beakers used as test vessels which are then filled with clean seawater. Five replicate containers for each test sediment, reference sediment, and the control sediment, each containing 5 test organisms, are employed. During test setup, three subsamples of five worms each are randomly selected to provide an estimate of initial biomass. During the exposure period, each chamber is provided with 40 mg of food on an every-other-day basis. Every third day, one-third of the seawater in each chamber is exchanged with fresh seawater. Survival and growth are the response criteria used. The mean and standard deviation for each treatment and test endpoint are given in the final report. Between-treatment statistical comparisons may be made, where each treatment is compared to the control and/or reference sediment. This protocol is based on PSEP (1995) guidelines, PSDDA (PSDDA 1989, U.S. ACOE et al. 2000) modifications, and SMS guidelines (WDOE 2003).

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

2.2 Sponsor's Study Monitor:

2.3 Name of Testing Laboratory:

Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location: Newport Laboratory

2.5 Laboratory's Personnel to be Assigned to the Study:

Project Manager/Technical Director: _____
Qual. Assurance Officer: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: Tests should begin within 2 weeks (8 weeks with samples under nitrogen for PSDDA or SMS) of sample collection. Reference toxicant test to be run concurrently.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are marine or estuarine sediments. The collected sediments are placed in a suitable container for shipping and storage. The preferred container is a solvent and acid cleaned 1 L glass jar fitted with a TFE-lined screw cap. The jars are filled completely so that there is no air space. At the laboratory, the samples may be stored at 4°C in the dark in the original sealed containers for up to 2 weeks (8 weeks with no headspace or with samples under nitrogen for PSDDA or SMS) prior to testing. The negative control sediment is from a clean site. In addition, one or more reference sediments, clean sediments with physical characteristics similar to the test sediments, may also be employed.

4. TEST WATER

Test water is filtered Yaquina Bay seawater adjusted to a salinity of 28 ppt. The water is pumped from Yaquina Bay into a 6000 gal seasoned fiberglass reservoir from which it is supplied under pump pressure to the laboratory. Filtration is accomplished using a sand filter followed by 5 µm, 1µm, and 0.40µm cartridge filters. An alternative seawater supply of similar quality may be used.

5. TEST ORGANISMS

5.1 Species: *Neanthes arenaceodentata*

5.2 Source: *Neanthes* are obtained from laboratory cultures. California State University, Long Beach, Dept. of Biology is a source for purchasing these worms.

5.3 Laboratory Handling: Worms are received from the supplier in plastic bags containing seawater and algae (*Enteromorpha*). Upon receipt, water quality data are taken on 1 or 2 bags and all bags with worms are then placed in holding aquaria containing seawater at $20 \pm 1^\circ\text{C}$, and the salinity is adjusted to 28 ± 2 ppt. Worms are generally maintained for 1-2 days prior to use in tests, but may be held longer if water changes are provided. Gentle aeration is supplied to the holding aquaria. During the holding period, organisms are fed ground TetraMarine® (approximately 0.1 g per 200-300 worms) on an every-other-day basis. If the food is not being consumed, feeding should be reduced. No water changes are required if holding time is less than 1 week.

5.4 Age at Study Initiation: 2-3 week post-emergence juveniles (0.5-1.0 mg dry weight).

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 1000 ml glass beakers. The beakers are covered to minimize contamination and evaporation of seawater or loss of volatile compounds. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Minimal aeration is supplied through a glass pipet. The aeration rate should be 150 - 300 mL/minute or approximately 100 bubbles per minute). The test is performed under continuous illumination, using ambient laboratory lighting of low to moderate intensity. SMS may require UV light for PAH-contaminated intertidal sediments.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in an automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times more with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: An experimental design is used consisting of exposure of test organisms to a number of test sediments, one or more reference sediments, and a control sediment. Each treatment consists of five replicate test chambers each containing 5 animals. An additional replicate containing 5 test organisms is used for daily water quality measurements. More replicates, with or without test organisms as appropriate, may be employed for periodic interstitial water quality measurements. Blind, random testing is used.

- 7.2 Preparation of Test Sediments: The interstitial salinity of the test, reference, and control sediments is measured. If initial interstitial salinities are not ≥ 20 ppt, the interstitial salinities must be adjusted as specified in the PSEP (1995) protocols.

Certain projects may require purging of excess ammonia from sediment interstitial water. PSDDA and SMS allow purging to be considered when interstitial total ammonia concentrations exceed specified limits. However, purging is allowed only by agency permission, so the decision to purge should be made by the client. Testing of purged sediments may require concurrent testing of the same sediments unpurged.

If no salinity adjustment or purging is done, sediments are used without further treatment. Each test sediment is mixed thoroughly using a non-contaminating implement, then an aliquot (175 ml) sufficient to make a 2-cm-deep layer is added to each test beaker, and the surface is smoothed. Bubbles are removed from the sediment by gently tapping each beaker against the palm of the hand. Seawater at the test temperature and salinity is carefully added into the beaker to the 750 ml mark utilizing a water dispersal technique to avoid suspending the sediment. The beakers are then placed into the water bath or constant-temperature room and covered with watchglasses. An air delivery pipet is inserted into each beaker under the watchglass. Overhead lights provide constant illumination. Water in the test beakers is aerated without disturbing the sediments. The test system is then allowed to temperature equilibrate overnight.

- 7.3 Beginning the Test: More worms than are needed for the bioassay are transferred to a shallow glass dish containing seawater of the test salinity and temperature. Worms are impartially distributed to a series of seawater-filled cups, each holding 5 worms. Enough cups for the test plus three additional cups are prepared. One container of 5 worms is randomly added to each replicate. Three of the cups containing worms are randomly selected and set aside. Worms from these cups are used to estimate initial total biomass. To determine initial total biomass, worms from these three cups are quickly rinsed with deionized water, placed on a preweighed aluminum pan, dried at 50°C to a constant weight, and weighed to the nearest 0.1 mg.

Once worms are added to a replicate, the number of animals that do not burrow into the test sediment within one hour is recorded. Worms not burrowed are removed and replaced with healthy worms, unless the observer believes the failure to burrow is a response to toxic material. Following addition of worms to the test chambers, additional water is added to achieve a final volume of 950 ml.

TetraMarine[®] is provided to each beaker (40 mg/beaker). The food is preweighed into plastic cups, wetted with bioassay test water, and rinsed into the test beakers.

- 7.4 Effects Criteria: Effects criteria are 1) survival after 20 days, 2) total biomass (dry weight), 3) average individual biomass (total biomass divided by the number of surviving worms), and 4) average individual growth rate.
- 7.5 Test Conditions: Test containers are maintained at a constant $20 \pm 1^\circ\text{C}$. The test salinity is 28 ± 2 ppt. The dissolved oxygen concentration in each test container must be greater than 60% saturation (PSEP 1995) or 4.0 mg/L (PSDDA) throughout the 20-day test. Each beaker is supplied with oil-free compressed air provided at a rate of approximately 100 bubbles per minute through disposable glass pipettes. Each beaker is covered by a watchglass to minimize evaporation and the possibility of cross contamination between beakers. The test is conducted under constant illumination of moderate to low intensity.

During the 20-day exposure, one-third of the seawater in each beaker is replaced every third day. Prior to seawater replacement, water quality measurements are determined for one replicate test chamber for each test, reference, and control sediment. Water replacement is achieved by siphoning one-third of the volume of test water, then replacing it with fresh 28 ± 2 ppt test water that has been maintained at $20 \pm 1^\circ\text{C}$. Care should be taken not to disturb the sediments.

- 7.6 Feeding: Animals are fed 40 mg TetraMarine[®] per beaker on an every-other-day basis during the test.

- 7.7 Test Duration, Type and Frequency of Observations, and Methods: The duration of the sediment toxicity test is 20 days. The test chambers are observed daily to ensure that adequate aeration is provided and to note the general status of each chamber. The temperature of the environmental chamber should also be monitored daily. The type and frequency of observations to be made are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION
Biological Data	
Survival	end of test
Biomass	end of test
Physical and Chemical Data	
Sediment interstitial salinity	at test beginning (bulk sediments) & end
Salinity, dissolved oxygen, pH, and temperature of overlying water (one replicate only)	day 0, day 20 and prior to partial replacement of test water every third day
Temperature in temperature beaker	daily
Ammonia and sulfides in overlying water (one replicate)	at test beginning & end; for PSDDA and SMS, on day 3 prior to seawater replacement *
Ammonia, sulfide, pH & salinity in interstitial water (optional)	for PSDDA and SMS, ammonia, pH, & salinity in bulk sediments; additional measurements as requested by client
Check air and lights	daily

*PSEP (1995) recommends ammonia-N and sulfide monitoring prior to the first and second water renewals when initial monitoring reveals >0.7 mg/L un-ionized ammonia or >5.0 mg/L sulfides.

Following the exposure period, worms from each chamber are removed from the test sediment by gently sieving the sediment through a 0.5 mm screen. Worms often are in their tubes and can be removed by gently prodding either end of the tube with a fine-point paintbrush. The number of surviving worms is recorded. Surviving worms are placed in a container of clean seawater until all chambers have been sieved. Then worms from each chamber are quickly rinsed with deionized water, placed on a pre-weighed aluminum pan, dried at 50°C to a constant weight, and weighed to the nearest 0.1 mg.

Dissolved oxygen is measured directly in test bakers using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a properly calibrated pH meter with scale divisions of 0.1 pH units. Temperature is measured using a calibrated mercury thermometer or a telethermometer. Salinity is measured using a refractometer. The method used for the measurement of total ammonia-N in the overlying water and sediment porewater from sediment bioassays is based on the salicylate colorimetric method of Hach Chemical Co. and was adapted from Clin. Chim. Acta., 14: 403 (1966). The method used for the measurement of dissolved sulfide in the overlying water and sediment porewater in marine sediment bioassays is the methylene blue colorimetric method based on SM 4500-S²⁻ (Standard Methods 1995 [19th edition]). Any observed changes in sediment color or the formation of a sediment discontinuity layer is also recorded.

- 7.8 Criteria of Test Acceptance: For the test to be considered acceptable, mean mortality of organisms in the control treatment at the end of the test should be ≤10%
- 7.9 Performance criteria: For PSDDA and SMS, control sediment should have mean mortality ≤10% and target mean growth rate >0.72 mg/worm/day (failure if growth rate <0.38 mg/worm/day). Initial weight of worms should be 0.5-1.0 mg (failure if <0.25 mg). Reference sediment should have mean mortality ≤20% and mean growth rate ≥80% of the control sediment.
- 7.10 Reference Toxicant test: A routine reference toxicant test is run concurrently with each sediment test. This is normally a 96-hr test with cadmium chloride. PSDDA and SMS also require a water-only ammonia test when ammonia concentrations exceed specified limits.

8. DATA ANALYSIS

The mean and standard deviation are calculated for each endpoint employed (e.g. 20-day survival, 20-day total biomass, 20-day average individual biomass, and average individual growth rate) and for each treatment (i.e. test sediment). Between-treatment comparisons may be made using a Student's t-test, Wilcoxon's Two-Sample test, Mann-Whitney U test, or Rankit Analysis, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons.

9. REPORTING

A report of the test results must include the following information: name and identification of the test; the investigator and laboratory; information on the test, reference, and control sediments including the initial and final interstitial salinities; information on the source of seawater used; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including water quality; information about any aeration that may have been required; definition of the effect criteria and other observations; unusual responses, if any, in the control treatment; 20-day survival in each exposure chamber and the mean and standard deviation for each treatment; initial total biomass (dry weight) for three groups of five worms; 20-day total biomass (dry weight) in each exposure chamber and the mean and standard deviation for each treatment; 20-day average individual biomass (dry weight) in each exposure chamber and the mean and standard deviation for each treatment; average individual growth rate in each exposure chamber and the mean and standard deviation for each treatment; 96-hour LC50 with reference toxicant; a description of data analysis methods employed and documentation of statistical test results; any unusual information about the test or deviations from procedures.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect, and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCED PROCEDURES

Johns, D.M., T.C. Ginn, and D.R. Reish. 1990. Protocol for juvenile Neanthes sediment bioassay. Prepared for U.S. Env. Prot. Agency, Region 10 -Office of Puget Sound, Seattle, WA, 17 pp.

Puget Sound Dredged Disposal Analysis (PSDDA). 1989. Management plan report - unconfined open-water disposal of dredged material, Phase II - (north and south Puget Sound). Puget Sound Dredged Disposal Analysis, Army Corps of Engineers, Seattle, WA. (and other modifications made through the PSDDA process and Sediment Management Annual Review Meetings).

Puget Sound Estuary Program. 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound sediments. Prepared for U.S. Env. Prot. Agency, Region 10, Office of Puget Sound, Seattle, WA, and Puget Sound Water Quality Authority, Olympia, WA.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods. Sixth Ed., The Iowa State Univ. Press. Ames, Iowa., 593 pp.

U.S. Army Corps of Engineers, Seattle District; U.S. EPA, Region 10; Washington Department of Natural Resources; Washington Department of Ecology. 2000. Dredged material evaluation and disposal procedures: a user's manual for the dredged disposal analysis (PSDDA) program.

Washington State Department of Ecology. 2003. Sediment sampling and analysis plan appendix. Publication no. 03-09-043, WDOE, Olympia, WA.

Weber, C.I. (Ed.) 1991. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms (fourth edition). EPA/600/4-90/027.

12. APPROVALS

_____ for _____
Name Date

_____ for NORTHWESTERN AQUATIC SCIENCES
Name Date

APPENDIX II

RAW DATA

**TEST DESCRIPTION, MONITORING, AND RESULTS
BENCHSHEETS**

NORTHWESTERN AQUATIC SCIENCES
NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

PROTOCOL NO. NAS-XXX-NA4
REVIEWED
PAGE 1-45
-631

Test No. 865-2 Client Anchor QEA Investigator

STUDY MANAGEMENT

Client: ANCHOR QEA, LLC, 720 Olive Way, Suite 1900, Seattle, WA 98101

Client's Study Monitor: Ms. Cindy Fields

Testing Laboratory: Northwestern Aquatic Sciences

Test Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Man./Study Dir. G. J. IRISSARRI 631

QA Officer L.K. Nemeth

1. Y. Veredagharu 12 2. Labaker 015

3. J. Brown 13 4. R.S. Caldwell, PSC

5. Lauren Brady 15 6.

7. 8.

Study Schedule:

Test Beginning: 3-31-15 1040 Test Ending: 4-20-15 1130

TEST MATERIAL

General description (see sample logbook/chain-of-custody for details):

NAS Sample No.:	5231G	5245G	5246G	5247G	5230G
Description:	WWD05-0-4-150208	WWD02-4-8-150205	WWD02-0-4-150205	WWD12-0-4-150208	WWD-REF-150319
Collection Date:	2/6/2015	2/5/2015	2/5/2015	2/6/2015	3/19/2015
Receipt Date:	3/23/2015	3/23/2015	3/23/2015	3/23/2015	3/20/2015
Inters.Salinity (ppt):	29.0	29.5	29.5	29.0	27.0

NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					

NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					

NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					

Test No.	865-2	Client	Anchor QEA	Investigator
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SEDIMENT DESCRIPTIONS -- SUPPLEMENTAL NOTES

[illegible]

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-2 Client Anchor QEA Investigator **TEST WATER**Source: Yaquina Bay, Oregon, sea waterDate(s) of Collection: 3-25-15 Salinity (ppt) 25.0 pH 8.0Treatments: filtered to ≤ 0.45 μ m, salinity-adjusted with MilliQ deionized water, aerated**TEST ORGANISMS**Species: *Neanthes arenaceodentata*Date received: 3-27-15Source: Aquatic Toxicology Support, Bremerton, WA**Acclimation Data:**

Date	Temp. (deg.C)	pH	Sal (ppt)	DO (mg/L)	Feeding		Water changes	Comments
					amount	description		
3-27-15	17.3	7.2	28.0	7.3		TETRA MARINE	YES	
3-28-15	20.7	7.9	28.0	7.2		—	—	
3-29-15	20.5	8.0	29.0	7.5		TETRA MARINE	YES	
3-30-15	20.6	7.9	28.0	7.2		—	YES	
3-31-15	20.5	8.0	27.0	7.3		—	—	
Mean	19.9	7.8	28.0	7.3				
S.D.	1.5	0.3	0.7	0.1				
(N)	5	5	5	5				

Photoperiod during acclimation: CONSTANT LIGHT**TEST PROCEDURES AND CONDITIONS**

Test chambers: 1 L glass beakers covered with watchglasses

Test volumes: 175 ml of test sediment; 950 ml total volume

Replicates/treatment: (5) 5 Organisms/treatment: (25) 25 (5/REP)

Test water changes: Every third day beginning with day 3

Aeration: yes, at least 2 cm above sediment surface

Beaker placement: Total randomization

Feeding: every other day beginning with day zero

Photoperiod: Constant light

Test temperature (deg.C): 20 \pm 1 deg CSalinity: 28 \pm 2 ppt

Additional replicates included for water quality purposes (indicate numbers of each that apply):

☒ Water quality beaker (with test organisms; may also be day 20 sacrificial beaker)☐ Day zero sacrificial beaker (no test organisms)☐ Day sacrificial beaker(s) (with test organisms)**Control Sediment:**Source: Yaquina Bay, OregonDate collected: 3-25-15 Interstitial salinity: 22.0 pptSieved through 0.5 -mm screenStorage: 4°C in the dark NAS# 52506**MISCELLANEOUS NOTES**

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-2 Client _____ Anchor QEA _____ Investigator _____Test conducted in (circle one): room 1 room 2 trailer water bath other: _____

Randomization chart:

3	6	9	12	15	18	21	24	27	30
2	5	8	11	14	17	20	23	26	29
1	4	7	10	13	16	19	22	25	28

Randomization chart:

33	36								
32	35								
31	34								

Randomization chart:

Randomization chart:

TEST SCHEDULE SUMMARY

Date	3/31/15	4-1-15	4-2-15	4-3-15	4-4-15	4-5-15	4-6-15	4-7-15	4-8-15	4-9-15	4-10-15	4-11-15	4-12-15	4-13-15	4-14-15	4-15-15	4-16-15	4-17-15	4-18-15	4-19-15	4-20-15
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Feed	x		x		x		x		x		x		x		x		x		x		
Record WQ	x			x			x			x			x			x			x		x
Change water				x			x			x			x			x			x		
Overlying NH ₄ S	x			NH ₃																	x
Initials	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS	JS
Porewater NH ₄ , ppt, pH																					ppt only
Initials	JS																				JS

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-2 Client _____ Anchor QEA _____ Investigator _____

DAILY RECORD SHEET

Day 0 3/11/15 OB/LATemperature beaker: 20.4 deg.Cair okay? (if not, details in comments below) YESoverlying ammonia sampled? yeswater change? (every third day) --

(days 0,3,20)

fed? (every second day) yes

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	S (mg/L)	No. not buried	Comments
1						0	
2						0	
3						0	
4						0	
5						0	
6						0	
7						0	
8						0	
9						0	
10						0	
11						0	
12						0	
13						0	
*14	20.3	8.2	28.0	7.3	0.02	0	
15						0	
16						0	
17						0	
*18	20.2	8.1	28.0	7.1	0.02	0	
19						0	
20						0	
21						0	
22						0	
23						0	
24						0	
*25	20.4	8.0	28.5	6.9	<0.02	0	
26						0	
27						0	
28						0	
*29	20.3	8.2	28.0	7.3	<0.02	0	
*30	20.3	8.2	28.0	7.3	<0.02	0	
*31	20.4	8.1	28.0	7.1	<0.02	0	
32						0	
33						0	
34						0	
35						0	
36						0	

* Water quality beakers

Test No.	865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 1 (4/1/15) 093 Temperature beaker: 20.3 deg.C

air okay? (if not, details in comments below) yes

water change? (every third day) —

fed? (every second day) —

[illegible]

Day 2 (4/2/15) ✓ Temperature beaker: 20.0 deg.C

air okay? (if not, details in comments below) *YES*

water change? (every third day) 1

fed? (every second day) Yes

[illegible]

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Test No.	865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 3 (14/3/15) re /x

Temperature beaker: 20.3 deg.C

air okay? (if not, details in comments below) YES

overlying NH₃ sampled (days 0,3,20)? 14 Ys

water change? (every third day) YES

fed? (every second day) —

[illegible]

Day 4 (4 M 1575)

Temperature beaker: 20.1 deg.C

air okay? (if not, details in comments below) 42

water change? (every third day) —

fed? (every second day) 42

[illegible]

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Day 5 (415/115) 6M

Temperature beaker: 20.1 deg.C

water change? (every third day) —

fed? (every second day) —

Day 6 (4/16/15) LB

Temperature beaker: 200 deg.C

water change? (every third day) ✓ 13

fed? (every second day) YES

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DAILY RECORD SHEET

Day 7 (4/7/15) ✓

Temperature beaker: 20.0 deg.C

air okay? (if not, details in comments below) YES
 water change? (every third day) —
 fed? (every second day) —

Day 8 (4/8/15) Temperature beaker: 20.1 deg.C

air okay? (if not, details in comments below) yes
 water change? (every third day) -
 fed? (every second day) yes

Page 9 of 45

Test No.	865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 9 (419115)-✓

Temperature beaker: 20.0 deg.C

air okay? (if not, details in comments below) *YES*

water change? (every third day) yes

fed? (every second day) —

[illegible]

Day 10 (4 110 115) ✓

Temperature beaker: 20.2 deg.C

air okay? (if not, details in comments below) YES

water change? (every third day)

fed? (every second day) YES

[illegible]

Test No.	865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 11 (4 11 15) *OR* Temperature beaker: 20.1 deg.C

air okay? (if not, details in comments below) *yes*

water change? (every third day) —

fed? (every second day) —

[illegible]

Day 12 (4/12/15) ✓

Temperature beaker: 19.9 deg.C

air okay? (if not, details in comments below) YES

water change? (every third day) YES

fed? (every second day) YES

[illegible]

Test No.	865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 13 (4/13/15) ✓

Temperature beaker: 20.1 deg.C

air okay? (if not, details in comments below) YES

water change? (every third day)

fed? (every second day) —

[illegible]

Day 14 (4/14/15) WS

Temperature beaker: 20.3 deg.C

air okay? (if not, details in comments below) ✓

water change? (every third day) —

fed? (every second day) \sqrt{cs}

[illegible]

Test No.	865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 15 (4 115 115) LB

Temperature beaker: 20-1 deg.C

air okay? (if not, details in comments below) YES

water change? (every third day) ✓

fed? (every second day) —

[illegible]

Day 16 (4/16/15) ✓

Temperature beaker: 20.3 deg.C

air okay? (if not, details in comments below) *yes*

water change? (every third day)

fed? (every second day) yes

[illegible]

Test No. 865-2	Client	Anchor QEA	Investigator
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DAILY RECORD SHEET

Day 19 (4/19/15) 60%

Temperature beaker: 20.1 deg.C

air okay? (if not, details in comments below) yes

water change? (every third day)

fed? (every second day) —

[illegible]

Day 20 (4/20/15) -

Temperature beaker: 20.1 deg.C

air okay? (if not, details in comments below) *yes*

water change? (every third day)

fed? (every second day) _____

overlying NH3 sampled (days 0,3,20)? YES

[illegible]

Investigator

DAY 20 TEST TERMINATION SHEET

[illegible]

* Water quality beakers

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-2Client Anchor QEAInvestigator

ZERO-TIME WEIGHING DATA SHEET

Ashed

tare:

Date 3-31-15Oven temp (C.) 550

Drying time

(hr.) 2Initials JRF

Standard Weights:

10 mg: 10.009100mg: 100.019

Final:

Date 4-1-15Oven temp (C.) 51

Drying time

(hr.) 24Initials JRF

Standard Weights:

10 mg: 10.006100mg: 100.018

Ashed

Final:

Date 4-2-15Oven temp (C.) 550

Drying time

(hr.) 2Initials JRF

Standard Weights:

10 mg: 10.007100mg: 100.019

Equip. used:

Oven: Blue M #2Balance: Sartorius M3P

(Dry overnight at 50C for dry weight, then 2 hrs at 550C for ashed weight)

Pan #	Tare	Final		#weighed	Comments
	Ashed tare wt. (mg)	Total wt. (mg)	Ashed total wt. (mg)		
1	44.46	46.70	44.64	5	
2	41.83	44.16	42.02	5	
3	43.67	46.34	43.90	5	

NEANTHES SP. 20-DAY SOLID PHASE SEDIMENT TEST

Test No. 865-2 Client _____ Anchor QEA _____ Investigator _____

WEIGHING DATA SHEET

Tare: Date 4-2-15 Oven temp (C.) 550 Drying time (hr.) 2 Initials JRF
 Standard Weights: 10 mg: 10.069 100mg: 100.017

Final #1: Date 4-22-15 Oven temp (C.) 52 Drying time (hr.) 24 Initials JRF
 Standard Weights: 10 mg: — 100mg: —

Final #2: Date 4-22-15 Oven temp (C.) 52 Drying time (hr.) 2 Initials JRF
 Standard Weights: 10 mg: — 100mg: —

Final #3: Date 4-23-15 Oven temp (C.) 53 Drying time (hr.) 21 Initials JRF
 Standard Weights: 10 mg: — 100mg: —

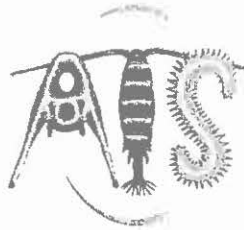
Ashed wt: Date 4-27-15 Oven temp (C.) 550 Drying time (hr.) 2 Initials JRF
 Standard Weights: 10 mg: — 100mg: —

Equip. used: Oven: BLUE M^{H2}, FISHER ISOTHERM MUFFLE FURNACE Balance Sartorius M3P

(Dry overnight at 50C [final #1], 1-2 hrs at 50C [finals #2 & #3], 2 hrs at 550C [ashed wt.])

Bkr. #	Pan #	Tare wt. (mg)	Total dry wt. (mg)			no. weighed	into pans -initials	Ashed wt. (mg)	Comments
			1	2	3				
1	1	102.90	165.44		164.82	5	LB	119.11	
2	2	101.44	191.46		190.74	5	LB	123.79	
3	3	105.13	169.21		168.71	5	LB	122.40	
4	4	99.88	182.61		181.70	5	LB	117.18	
5	5	96.07	162.75		162.20	5	LB	108.94	
6	6	98.52	180.63		180.07	5	LB	117.60	
7	7	94.68	164.60		164.08	5	LB	114.75	
8	8	98.88	164.92		164.31	5	LB	116.07	
9	9	100.10	138.82		183.46	4	LB	108.65	
10	10	92.93	171.40		170.38	5	LB	110.49	
11	11	69.22	124.56		124.11	4	LB	76.98	
12	12	97.47	146.10		145.65	5	LB	106.20	
13	13	102.54	195.05		193.84	5	LB	128.56	
14	14								
15	15	102.19	157.57		156.85	5	LB	116.05	
16	16	95.14	146.81		146.17	4	LB	102.93	
17	17	104.27	166.65	166.39	165.93	5	LB	119.20	
18	18								
19	19	100.92	184.91	184.57	^{JP} 184.189.97	5	LB	118.73	
20	20	101.72	176.39	176.11	175.59	5	LB	118.01	
21	21	94.79	147.34	147.11	146.79	4	LB	103.08	
22	22	105.12	159.04	158.82	158.59	3	LB	117.57	
23	23	102.09	157.31	157.12	156.86	5	LB	114.49	
24	24	103.98	167.99		167.38	5	LB	118.02	
25	25								
26	26	90.42	154.62		153.93	4	LB	104.44	
27	27	98.38	144.36	143.5 ^{JP}	143.79	5	LB	109.07	
28	28	94.77	153.69		153.12	4	LB	111.32	
29	29								
30	30								

* Water quality beakers



Recd 3-27-15

Aquatic Toxicology Support
1849 Charleston Beach Road West
Bremerton, Washington 98312
(360) 813-1202

Order Summary

Species: <i>Neanthes arenaceodentata</i> *	Emerge Date: 9-11 March '15
Number Ordered: 390	Number Shipped: 390 + 10%
Date Shipped: 26 March '15	Salinity (ppt): 30

*Smith 1964. CSU Long Beach strain. Feed upon arrival.

TEST DATA ANALYSIS RECORDS

data entry verified against
laboratory bench sheets 5-11-15
JZF

ENDPOINTS DATA ENTRY AND CALCULATIONS FILE

BKR=beaker number (=pan number)

INIT NO =initial number of worms exposed

FINAL IW PPT=interstitial salinity in ppt on day 20

SURV=number of worms surviving after 20 days

TARE WT=ashed weight of pan on day 20 (mg)

WT COUNT=number of worms weighed at least end

FINAL DRY WT=TARE WT + dry weight of worms

recovered on day 20 (mg)

PSURV=% SURV=100(SURV/INIT NO.)

PMORT=%MORT=100((INIT NO.-SURV)/INIT NO.)

TWT=total biomass=FINAL DRY WT-TARE WT

WT=average individual biomass=TWT/WT COUNT

GR=individual growth rate=(WT-INIT WT)/20

ASHED DRY WT= weight of ashed pan + weight of ashed

material recovered at test termination (mg)

TAFDW=FINAL DRY WT - ASHED DRY WT= total ash-free

biomass for given replicate (mg)

AFDW=average individual ash-free biomass=TAFDW/WT COUNT

AFDW GR = average individual growth rate on an ash-free-dry-weight basis

= (AFDW - INIT AFDW)/20

INITIAL WEIGHTS

TABLE 1								
pan#	tare wt	final wt	tare	#	weighed wt	individual ashed wt	afdw	ash-free individual wt
1	44.46	46.70	2.24	5	0.45	44.64	2.06	0.412
2	41.83	44.16	2.33	5	0.47	42.02	2.14	0.428
3	43.67	46.34	2.67	5	0.53	43.90	2.44	0.488
MEANS			2.41		0.48		2.21	0.443

INDEX	BKR	NAS SMPL	CLIENT DESCRIP	REPL	INIT NC	FINAL IW PPT	SURV	FINAL DRY WT	TARE WT	WT COUNT	ASHED DRY WT	PSURV	PMORT	TWT	WT	GR	TAFDW	AFDW	AFDW GR	PSURV	PMORT	TWT	WT	GR	AFDW	AFDW GR	
1	7	5250G	Control	1	5		5	184.08	94.88	5	114.75	100.0	0.0	89.4	13.9	0.87	48.33	9.87	0.47								
2	27	5250G	Control	2	5		5	143.79	98.38	5	109.07	100.0	0.0	45.4	9.1	0.43	34.72	8.94	0.33	Mean	100.0	0.0	60.5	12.1	0.58	9.2	0.44
3	1	5250G	Control	3	5		5	184.82	102.90	5	119.11	100.0	0.0	81.9	12.4	0.80	45.71	9.14	0.43	S.D.	0.0	0.0	13.7	2.7	0.14	1.9	0.10
4	10	5250G	Control	4	5		5	170.38	92.93	5	110.48	100.0	0.0	77.5	15.5	0.75	59.89	11.98	0.58	n	5	5	5	5	5	5	
5	12	5250G	Control	5	5		5	145.85	97.47	5	108.20	100.0	0.0	48.2	9.8	0.48	39.45	7.89	0.37								
6	30	5250G	Control	6	WO	5	29.5																				
7	8	5248G	WWD02-0-4-150205	1	5		5	180.07	98.52	5	117.60	100.0	0.0	81.6	16.3	0.79	82.47	12.49	0.60	Mean							
8	22	5248G	WWD02-0-4-150205	2	5		5	158.59	105.12	3	117.57	80.0	40.0	53.5	17.8	0.87	41.02	13.87	0.66	S.D.	88.0	12.0	87.9	15.8	0.75	12.3	0.59
9	11	5248G	WWD02-0-4-150205	3	5		5	124.11	89.22	4	78.98	80.0	20.0	54.9	13.7	0.88	47.13	11.78	0.57	n	17.9	17.9	14.1	2.0	0.10	1.2	0.08
10	19	5248G	WWD02-0-4-150205	4	5		5	183.97	100.82	5	118.73	100.0	0.0	83.1	16.8	0.81	65.24	13.05	0.83		5	5	5	5	5	5	
11	35	5248G	WWD02-0-4-150205	5	5		5	188.82	102.07	5	115.47	100.0	0.0	86.6	13.3	0.84	53.15	10.83	0.51								
12	31	5248G	WWD02-0-4-150205	6	WO	5	29.5																				
13	38	5245G	WWD02-4-8-150205	1	5		5	184.73	104.94	5	118.88	100.0	0.0	59.8	12.0	0.57	45.77	9.15	0.44	Mean							
14	8	5245G	WWD02-4-8-150205	2	5		5	184.31	98.88	5	118.07	100.0	0.0	65.4	13.1	0.83	48.24	9.85	0.46	S.D.	98.0	4.0	70.9	15.0	0.73	11.7	0.57
15	15	5245G	WWD02-4-8-150205	3	5		5	159.85	102.19	5	116.05	100.0	0.0	54.7	10.9	0.52	40.80	8.18	0.39	n	8.9	8.9	15.7	4.3	0.22	4.3	0.22
16	13	5245G	WWD02-4-8-150205	4	5		5	193.84	102.54	5	128.58	100.0	0.0	91.3	18.3	0.89	65.28	13.08	0.83		5	5	5	5	5	5	
17	9	5245G	WWD02-4-8-150205	5	5		5	183.46	100.10	4	108.65	80.0	20.0	83.4	20.8	1.02	74.81	18.70	0.91								
18	29	5245G	WWD02-4-8-150205	6	WO	5	29.5																				
19	21	5231G	WWD05-0-4-150208	1	5		5	148.79	94.79	4	103.08	80.0	20.0	52.0	13.0	0.83	43.71	10.93	0.52	Mean	82.0	8.0	85.5	14.3	0.69	11.3	0.55
20	24	5231G	WWD05-0-4-150208	2	5		5	187.38	103.88	5	118.02	100.0	0.0	83.4	12.7	0.81	49.38	9.87	0.47	S.D.	11.0	11.0	10.7	1.7	0.09	1.3	0.08
21	32	5231G	WWD05-0-4-150208	3	5		5	184.88	98.18	5	111.67	100.0	0.0	88.7	13.3	0.84	53.19	10.64	0.51	n	5	5	5	5	5	5	
22	4	5231G	WWD05-0-4-150208	4	5		5	181.70	99.88	5	117.16	100.0	0.0	81.8	18.4	0.78	64.52	12.90	0.82		5	5	5	5	5	5	
23	26	5231G	WWD05-0-4-150208	5	5		5	153.93	90.42	4	104.44	80.0	20.0	83.5	15.9	0.77	49.49	12.37	0.80								
24	25	5231G	WWD05-0-4-150208	6	WO	5	30.0																				
25	20	5247G	WWD12-0-4-150208	1	5		5	175.59	101.72	5	118.01	100.0	0.0	73.9	14.8	0.71	57.58	11.52	0.55	Mean	100.0	0.0	74.0	14.8	0.72	11.2	0.54
26	3	5247G	WWD12-0-4-150208	2	5		5	168.71	105.13	5	122.40	100.0	0.0	83.8	12.7	0.81	48.31	9.28	0.44	S.D.	0.0	0.0	10.2	2.0	0.10	1.5	0.07
27	5	5247G	WWD12-0-4-150208	3	5		5	182.20	98.07	5	108.94	100.0	0.0	68.1	13.2	0.84	53.26	10.65	0.51	n	5	5	5	5	5	5	
28	2	5247G	WWD12-0-4-150208	4	5		5	180.74	101.44	5	123.78	100.0	0.0	89.3	17.9	0.87	66.95	13.39	0.85								
29	33	5247G	WWD12-0-4-150208	5	5		5	175.78	98.60	5	120.25	100.0	0.0	77.2	15.4	0.75	55.53	11.11	0.53								
30	18	5247G	WWD12-0-4-150208	6	WO	5	30.0																				
31	23	5230G	WWD-REF-150318	1	5		5	156.88	102.09	5	114.49	100.0	0.0	54.8	11.0	0.52	42.37	8.47	0.40	Mean	82.0	8.0	81.7	13.4	0.65	10.3	0.49
32	28	5230G	WWD-REF-150318	2	5		5	153.12	84.77	4	111.32	80.0	20.0	58.4	14.6	0.71	41.80	10.45	0.50	S.D.	11.0	11.0	12.3	2.2	0.11	1.5	0.07
33	17	5230G	WWD-REF-150318	3	5		5	185.93	104.27	5	119.20	100.0	0.0	81.7	12.3	0.59	48.73	9.35	0.45	n	5	5	5	5	5	5	
34	34	5230G	WWD-REF-150318	4	5		5	185.80	103.28	5	124.13	100.0	0.0	82.5	16.5	0.80	61.67	12.33	0.59								
35	18	5230G	WWD-REF-150318	5	5		5	148.17	95.14	4	102.93	80.0	20.0	51.0	12.8	0.61	43.24	10.81	0.52								
36	14	5230G	WWD-REF-150318	6	WO	5	29.5																				

Project Name: P865-2 Neanthes Growth Rate (dry wt)

Sample: x1
 Samp ID: WWD02-0-4-150205
 Alias: NAS# 5246G
 Replicates: 5
 Mean: 0.754
 SD: 0.1
 Tr Mean: 0.754
 Trans SD: 0.1

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 0.646
 SD: 0.11
 Tr Mean: 0.646
 Trans SD: 0.11

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.068 SS: 0.088 K: 5 b: 0.287 Alpha Level: 0.05 Calculated Value: 0.9364 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.083 Test Residual SD: 0.036 Ref. Residual Mean: 0.087 Ref. Residual SD: 0.05 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.145 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -1.6296 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.79	0.79	0.52	0.52	0.036	0.126			-0.126
2	0.87	0.87	0.71	0.71	0.116	0.064			-0.114
3	0.66	0.66	0.59	0.59	0.094	0.056			-0.094
4	0.81	0.81	0.8	0.8	0.056	0.154			-0.056
5	0.64	0.64	0.61	0.61	0.114	0.036			-0.036
6									0.036
7									0.056
8									0.064
9									0.116
10									0.154

Average individual growth rate (dry wt) in test sediment WWD02-0-4-150205 is not significantly less than that in the reference sediment (WWD-REF-150319) at $\alpha=0.05$.

- 631

Project Name: P865-2 Neanthes Growth Rate (dry wt)

Sample: x1
 Samp ID: WWD02-4-8-150205
 Alias: NAS# 5245G
 Replicates: 5
 Mean: 0.726
 SD: 0.218
 Tr Mean: 0.726
 Trans SD: 0.218

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 0.646
 SD: 0.11
 Tr Mean: 0.646
 Trans SD: 0.11

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.112 SS: 0.237 K: 5 b: 0.473 Alpha Level: 0.05 Calculated Value: 0.9406 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.183 Test Residual SD: 0.073 Ref. Residual Mean: 0.087 Ref. Residual SD: 0.05 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 2.4148 Critical Value: ≥ 1.860 Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$ Degrees of Freedom: 6 Experimental Alpha Level: 0.05 Calculated Value: -0.7342 Critical Value: ≥ 1.943 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.57	0.57	0.52	0.52	0.156	0.126			-0.206
2	0.63	0.63	0.71	0.71	0.096	0.064			-0.156
3	0.52	0.52	0.59	0.59	0.206	0.056			-0.126
4	0.89	0.89	0.8	0.8	0.164	0.154			-0.096
5	1.02	1.02	0.61	0.61	0.294	0.036			-0.056
6									-0.036
7									0.064
8									0.154
9									0.164
10									0.294

Average individual growth rate (dry wt) in test sediment WWD02-4-8-150205 is not significantly less than that in the reference sediment (WWD-REF-150319) at $\alpha=0.05$. -651

Project Name: P865-2 Neanthes Growth Rate (dry wt)

Sample: x1
 Samp ID: WWD05-0-4-150206
 Alias: NAS# 5231G
 Replicates: 5
 Mean: 0.688
 SD: 0.085
 Tr Mean: 0.688
 Trans SD: 0.085

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 0.646
 SD: 0.11
 Tr Mean: 0.646
 Trans SD: 0.11

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.064 SS: 0.077 K: 5 b: 0.265 Alpha Level: 0.05 Calculated Value: 0.9125 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.074 Test Residual SD: 0.021 Ref. Residual Mean: 0.087 Ref. Residual SD: 0.05 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.5576 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -0.6769 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.63	0.63	0.52	0.52	0.058	0.126			-0.126
2	0.61	0.61	0.71	0.71	0.078	0.064			-0.078
3	0.64	0.64	0.59	0.59	0.048	0.056			-0.058
4	0.79	0.79	0.8	0.8	0.102	0.154			-0.056
5	0.77	0.77	0.61	0.61	0.082	0.036			-0.048
6									-0.036
7									0.064
8									0.082
9									0.102
10									0.154

Average individual growth rate (dry wt) in test sediment WWD05-0-4-150206 is not significantly less than that in the reference sediment (WWD-REF-150319) at $\alpha=0.05$. -631

Project Name: P865-2 Neanthes Growth Rate (dry wt)

Sample: x1
 Samp ID: WWD12-0-4-150206
 Alias: NAS# 5247G
 Replicates: 5
 Mean: 0.716
 SD: 0.102
 Tr Mean: 0.716
 Trans SD: 0.102

Ref Samp: x2
 Ref ID: Control
 Alias: NAS# 5250G
 Replicates: 5
 Mean: 0.582
 SD: 0.136
 Tr Mean: 0.582
 Trans SD: 0.136

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.078 SS: 0.116 K: 5 b: 0.33 Alpha Level: 0.05 Calculated Value: 0.9397 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.075 Test Residual SD: 0.058 Ref. Residual Mean: 0.11 Ref. Residual SD: 0.06 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.9216 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -1.758 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.71	0.71	0.67	0.67	0.006	0.088			-0.152
2	0.61	0.61	0.43	0.43	0.106	0.152			-0.122
3	0.64	0.64	0.6	0.6	0.076	0.018			-0.106
4	0.87	0.87	0.75	0.75	0.154	0.168			-0.076
5	0.75	0.75	0.46	0.46	0.034	0.122			-0.006
6									0.018
7									0.034
8									0.088
9									0.154
10									0.168

Average individual growth rate (dry wt) in test sediment WWD12-0-4-150206 is not significantly less than that in the control sediment at $\alpha=0.05$.

- 631

Project Name: P865-2 Neanthes Growth Rate (AFDW)

Sample: x1
 Samp ID: WWD02-0-4-150205
 Alias: NAS# 5246G
 Replicates: 5
 Mean: 0.594
 SD: 0.058
 Tr Mean: 0.594
 Trans SD: 0.058

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 0.492
 SD: 0.072
 Tr Mean: 0.492
 Trans SD: 0.072

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.042 SS: 0.034 K: 5 b: 0.182 Alpha Level: 0.05 Calculated Value: 0.9694 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.043 Test Residual SD: 0.032 Ref. Residual Mean: 0.054 Ref. Residual SD: 0.04 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.4582 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -2.4739 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.6	0.6	0.4	0.4	0.006	0.092			-0.092
2	0.66	0.66	0.5	0.5	0.066	0.008			-0.084
3	0.57	0.57	0.45	0.45	0.024	0.042			-0.042
4	0.63	0.63	0.59	0.59	0.036	0.098			-0.024
5	0.51	0.51	0.52	0.52	0.084	0.028			0.006
6									0.008
7									0.028
8									0.036
9									0.066
10									0.098

Average individual growth rate (AFDW) in test sediment WWD02-0-4-150205 is not significantly less than that in the reference sediment (WWD-REF-150319) at $\alpha=0.05$. -631

Project Name: P865-2 Neanthes Growth Rate (AFDW)

Sample: x1
 Samp ID: WWD02-4-8-150205
 Alias: NAS# 5245G
 Replicates: 5
 Mean: 0.566
 SD: 0.212
 Tr Mean: 0.566
 Trans SD: 0.212

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 0.492
 SD: 0.072
 Tr Mean: 0.492
 Trans SD: 0.072

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.103 SS: 0.201 K: 5 b: 0.425 Alpha Level: 0.05 Calculated Value: 0.8987 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.163 Test Residual SD: 0.109 Ref. Residual Mean: 0.054 Ref. Residual SD: 0.04 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 2.1158 Critical Value: ≥ 1.860 Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 5 Experimental Alpha Level: 0.05 Calculated Value: -0.7378 Critical Value: ≥ 2.015 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.44	0.44	0.4	0.4	0.126	0.092			-0.176
2	0.46	0.46	0.5	0.5	0.106	0.008			-0.126
3	0.39	0.39	0.45	0.45	0.176	0.042			-0.106
4	0.63	0.63	0.59	0.59	0.064	0.098			-0.092
5	0.91	0.91	0.52	0.52	0.344	0.028			-0.042
6									0.008
7									0.028
8									0.064
9									0.098
10									0.344

Average individual growth rate (AFDW) in test sediment WWD02-4-8-150205 is not significantly less than that in the reference sediment (WWD-REF-150319) at $\alpha=0.05$. -61

Project Name: P865-2 Neanthes Growth Rate (AFDW)

Sample: x1
 Samp ID: WWD05-0-4-150206
 Alias: NAS# 5231G
 Replicates: 5
 Mean: 0.544
 SD: 0.063
 Tr Mean: 0.544
 Trans SD: 0.063

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 0.492
 SD: 0.072
 Tr Mean: 0.492
 Trans SD: 0.072

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.044 SS: 0.037 K: 5 b: 0.188 Alpha Level: 0.05 Calculated Value: 0.963 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.053 Test Residual SD: 0.023 Ref. Residual Mean: 0.054 Ref. Residual SD: 0.04 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.0388 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -1.2123 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.52	0.52	0.4	0.4	0.024	0.092			-0.092
2	0.47	0.47	0.5	0.5	0.074	0.008			-0.074
3	0.51	0.51	0.45	0.45	0.034	0.042			-0.042
4	0.62	0.62	0.59	0.59	0.076	0.098			-0.034
5	0.6	0.6	0.52	0.52	0.056	0.028			-0.024
6									0.008
7									0.028
8									0.056
9									0.076
10									0.098

Average individual growth rate (AFDW) in test sediment WWD05-0-4-150206 is not significantly less than that in the reference sediment (WWD-REF-150319) at $\alpha=0.05$. -6J1

Project Name: P865-2 Neanthes Growth Rate (AFDW)

Sample: x1
 Samp ID: WWD12-0-4-150206
 Alias: NAS# 5247G
 Replicates: 5
 Mean: 0.536
 SD: 0.076
 Tr Mean: 0.536
 Trans SD: 0.076

Ref Samp: x2
 Ref ID: Control
 Alias: NAS# 5250G
 Replicates: 5
 Mean: 0.436
 SD: 0.097
 Tr Mean: 0.436
 Trans SD: 0.097

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 0.056 SS: 0.061 K: 5 b: 0.239 Alpha Level: 0.05 Calculated Value: 0.9411 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 0.051 Test Residual SD: 0.05 Ref. Residual Mean: 0.071 Ref. Residual SD: 0.055 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.6005 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.05 Calculated Value: -1.8161 Critical Value: ≥ 1.860 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	0.55	0.55	0.47	0.47	0.014	0.034			-0.106
2	0.44	0.44	0.33	0.33	0.096	0.106			-0.096
3	0.51	0.51	0.43	0.43	0.026	0.006			-0.066
4	0.65	0.65	0.58	0.58	0.114	0.144			-0.026
5	0.53	0.53	0.37	0.37	0.006	0.066			-0.006
6									-0.006
7									0.014
8									0.034
9									0.114
10									0.144

Average individual growth rate (AFDW) in test sediment WWD12-0-4-150206 is not significantly less than that in the control sediment at $\alpha=0.05$.

-631

Water Quality Data																	assumed interstitial temp	
BKR	NAS SMPL	CLIENT DESCRIP	REPL	DAY	TEMP	Overlying water					Interstitial							
						pH	SAL	DO	S	< or > NH4+NH3	< or > NH3	pH	SAL	Sulfide	< or > NH4+NH3	< or > NH3		
Bulk	5230G	WWD-REF-150319											7.5	27.0	0.2		7.8	0.097
Bulk	5231G	WWD05-0-4-150206											7.8	29.0	0.2		3.2	0.077
Bulk	5245G	WWD02-4-8-150205											8.1	29.5	<0.1		11.7	0.553
Bulk	5246G	WWD02-0-4-150205											8.0	29.5	<0.1		3.7	0.141
Bulk	5247G	WWD12-0-4-150206											8.0	29.0	<0.1		1.0	0.039
14	5230G	WWD-REF-150319	6	0	20.3	8.2	28.0	7.3	<0.02		1.3							
18	5247G	WWD12-0-4-150206	6	0	20.2	8.1	28.0	7.1	<0.02		0.1							
25	5231G	WWD05-0-4-150206	6	0	20.4	8.0	28.5	6.9	<0.02		0.1							
29	5245G	WWD02-4-8-150205	6	0	20.3	8.2	28.0	7.3	<0.02		0.7							
30	5250G	Control	6	0	20.3	8.2	28.0	7.3	<0.02		0.1		<	0.006				
31	5246G	WWD02-0-4-150205	6	0	20.4	8.1	28.0	7.1	<0.02		0.2							
14	5230G	WWD-REF-150319	6	3	20.0	8.0	28.5	6.7			1.8							
18	5247G	WWD12-0-4-150206	6	3	19.9	8.0	29.0	6.9			0.6							
25	5231G	WWD05-0-4-150206	6	3	20.3	7.9	28.0	6.7			0.7							
29	5245G	WWD02-4-8-150205	6	3	20.0	7.9	29.0	6.7			1.4							
30	5250G	Control	6	3	20.1	8.0	28.0	7.0			0.9							
31	5246G	WWD02-0-4-150205	6	3	20.2	7.9	28.5	6.5			1.0							
14	5230G	WWD-REF-150319	6	6	19.9	8.6	28.0	6.6										
18	5247G	WWD12-0-4-150206	6	6	19.6	8.2	29.0	7.1										
25	5231G	WWD05-0-4-150206	6	6	20.0	8.2	28.0	6.9										
29	5245G	WWD02-4-8-150205	6	6	19.6	8.1	28.0	7.0										
30	5250G	Control	6	6	19.9	8.2	28.0	7.2										
31	5246G	WWD02-0-4-150205	6	6	20.0	8.1	28.0	7.0										
14	5230G	WWD-REF-150319	6	9	20.0	8.2	29.5	6.7										
18	5247G	WWD12-0-4-150206	6	9	19.9	8.0	29.5	6.9										
25	5231G	WWD05-0-4-150206	6	9	20.0	8.0	29.0	6.9										
29	5245G	WWD02-4-8-150205	6	9	19.9	7.9	29.0	6.9										
30	5250G	Control	6	9	19.6	8.0	29.5	7.3										
31	5246G	WWD02-0-4-150205	6	9	19.9	7.9	29.5	6.7										
14	5230G	WWD-REF-150319	6	12	19.9	8.1	29.5	6.7										
18	5247G	WWD12-0-4-150206	6	12	19.7	8.0	30.0	6.5										
25	5231G	WWD05-0-4-150206	6	12	19.9	8.1	29.5	6.5										
29	5245G	WWD02-4-8-150205	6	12	19.6	8.0	29.5	6.5										
30	5250G	Control	6	12	19.6	8.0	29.5	6.5										
31	5246G	WWD02-0-4-150205	6	12	19.9	8.0	29.5	6.3										
14	5230G	WWD-REF-150319	6	15	20.2	7.9	28.0	6.2										
18	5247G	WWD12-0-4-150206	6	15	20.1	7.9	30.0	6.7										
25	5231G	WWD05-0-4-150206	6	15	20.1	7.9	29.5	6.4										
29	5245G	WWD02-4-8-150205	6	15	20.1	7.8	29.5	6.4										
30	5250G	Control	6	15	19.9	7.9	28.0	6.8										
31	5246G	WWD02-0-4-150205	6	15	20.1	7.9	28.0	6.1										
14	5230G	WWD-REF-150319	6	18	19.7	7.9	30.0	6.7										
18	5247G	WWD12-0-4-150206	6	18	19.5	7.9	30.0	6.6										
25	5231G	WWD05-0-4-150206	6	18	19.7	8.1	30.0	6.7										
29	5245G	WWD02-4-8-150205	6	18	19.6	8.0	29.5	6.5										
30	5250G	Control	6	18	19.5	7.9	30.0	6.6										
31	5246G	WWD02-0-4-150205	6	18	19.8	8.0	30.0	6.4										
14	5230G	WWD-REF-150319	6	20	19.5	7.9	29.0	6.5	<0.02		0.2						0.007	
18	5247G	WWD12-0-4-150206	6	20	20.0	7.9	30.0	6.7	<0.02		0.3						0.010	
25	5231G	WWD05-0-4-150206	6	20	20.1	8.1	29.5	6.7	<0.02		1.1						0.050	
29	5245G	WWD02-4-8-150205	6	20	19.9	8.0	29.5	6.9	<0.02		2.5						0.085	
30	5250G	Control	6	20	19.9	8.0	29.5	7.1	<0.02		1.5						0.056	
31	5246G	WWD02-0-4-150205	6	20	20.1	8.0	29.5	6.5	<0.02		0.3						0.013	
Mean						20.0	8.0	29.0	6.8				7.9	28.8				
SD						0.2	0.1	0.8	0.3				0.2	1.0				
n						48	48	48	48	12	18		5	5	5	5	5	
Min						19.5	7.8	28.0	6.1	<0.02	<0.1		7.5	27.0	<0.1	1.0	0.039	
Max						20.4	8.6	30.0	7.3	<0.02	2.5		8.1	29.5	0.2	11.7	0.553	

AMMONIA EXPOSURE BENCHSHEETS AND ANALYSIS

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

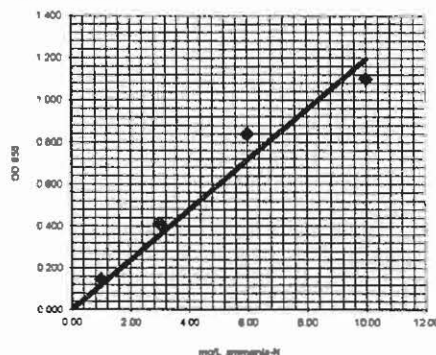
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449	3.71		
3.0 mg/L spike dupl.	----	0.444	3.67		
5.0 mg/L 2nd source		0.750	6.20		
1 5230G	5	0.189	7.81		
2 5231G	5	0.077	3.18		
3 5245G	5	0.283	11.70		
4 5246G	5	0.090	3.72		
5 5247G	5	0.025	1.03		

$$y = 0.1193x$$

$$R^2 = 0.9492$$

Standard Curve



Reporting limit (mg/L) = 0.50

Recovery (%) = 123.1

Precision (RPD) = 1.12

2nd source (%) = 124.0

Sample volume (ml): 0.10

Dilution factor 5

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)

X Bulk Sediment Porewaters

Test Beaker Porewaters

Overlying Water

Analyst:

RSC/JB

Date analysed:

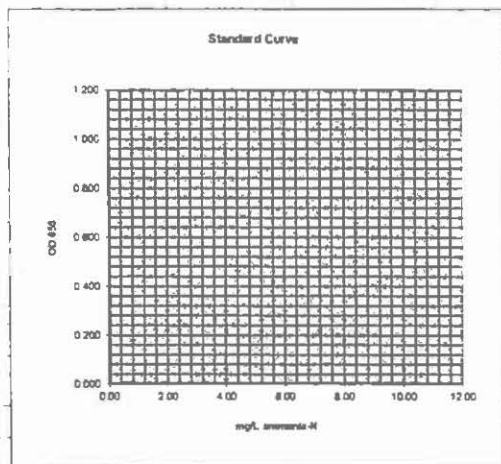
3/24/2015

Handwritten initials: RSC

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	---	---	---		
1.0 mg/L NH ₃ -N Std.	---	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	---	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	---	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	---	1.100	10.00		
3.0 mg/L spike	---	0.449			
3.0 mg/L spike dupl.	---	0.444			
5.0 mg/L 2nd source		0.750			
1 5230G	1	0.189		7.5	27.0
2 5231G	1	0.077		7.8	29.0
3 5245G	1	0.283		8.1	29.5
4 5246G	1	0.090		8.0	29.5
5 5247G	1	0.025		8.0	29.0



Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)

- ☒ Bulk Sediment Porewaters
☐ Test Beaker Porewaters
☐ Overlying Water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Handwritten initials: RSC/JB

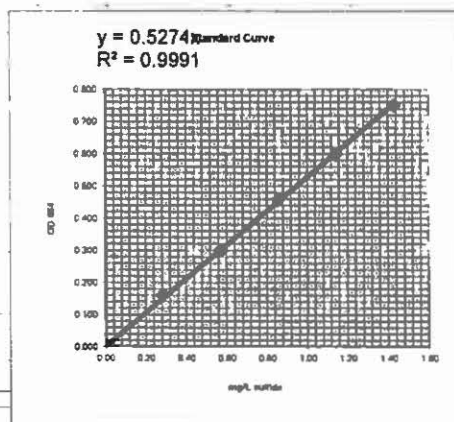
Dissolved Sulfide in Water: Computation Worksheet Methylene Blue Method (SOP #5550)

Standardization

	1	2	3
uL PAO titrant employed:	112	112	108
Working Std. Conc. (mg/L):		1.42933	

Result

Sample description	Dilution factor	OD ₆₆₄	Sulfide (mg/L)
Blank	-----	-----	-----
1.0 mL working sulfide std.	-----	0.161	0.29
2.0 mL working sulfide std.	-----	0.300	0.57
3.0 mL working sulfide std.	-----	0.460	0.86
4.0 mL working sulfide std.	-----	0.599	1.14
5.0 mL working sulfide std.	-----	0.751	1.43
3.0 mL spike	-----	0.451	0.86
3.0 mL spike dupl.	-----	0.450	0.85



1	5230G	5	0.025	0.24
2	5231G	5	0.021	0.20
3	5245G	5	0.005	ND
4	5246G	5	0.010	ND
5	5247G	5	0.000	ND

Reporting limit (mg/L) = 0.10

Recovery (%) = 99.6

Precision (RPD) = 0.22

Sample volume (ml): 1.00

Dilution factor 5

Sample Set Description:

Test No.:

Test Day:

Species:

Proj. No.: P865

X Bulk sediment porewaters

Test beaker porewaters

Overlying water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Dissolved Sulfide in Water: Computation Worksheet **Methylene Blue Method (SOP #5550)**

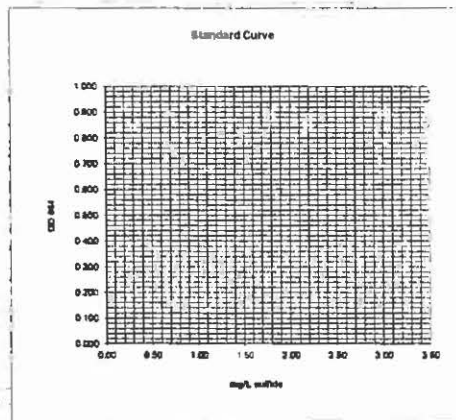
Standardization

uL PAO titrant employed:
 Working Std. Conc. (mg/L):

1	2	3
112	112	108
	3.2	

Result

Sample description	Dilution factor	OD ₆₆₄	Sulfide (mg/L)
Blank	---	---	---
1.0 mL working sulfide std.	---	0.161	0.64
2.0 mL working sulfide std.	---	0.300	1.28
3.0 mL working sulfide std.	---	0.460	1.92
4.0 mL working sulfide std.	---	0.599	2.56
5.0 mL working sulfide std.	---	0.751	3.20
3.0 mL spike	---	0.451	
3.0 mL spike dupl.	---	0.450	



1	5230G	5	0.025
2	5231G	5	0.021
3	5245G	5	0.005
4	5246G	5	0.010
5	5247G	5	0.000
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
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29			
30			
31			
32			
33			
34			
35			
36			

Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

Sample volume (ml): 1.00

Dilution factor 5

Sample Set Description:

Test No.:

Test Day:

Species:

Proj. No.: P865

X Bulk sediment porewaters

Test beaker porewaters

Overlying water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

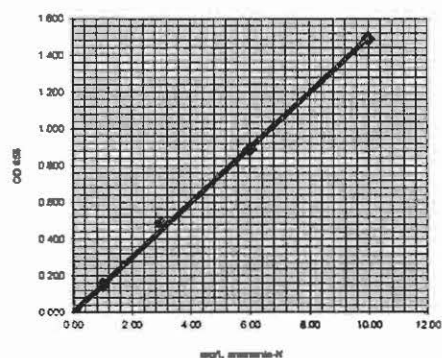
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.150	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.485	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.890	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.490	10.00		
3.0 mg/L spike	----	0.495	3.31		
3.0 mg/L spike dupl.	----	0.475	3.17		
5.0 mg/L 2nd source		0.700	4.68		
1 Day 0 (3-31-15)					
2 14	1	0.198	1.32		
3 18	1	0.017	0.11		
4 25	1	0.020	0.13		
5 29	1	0.100	0.67		
6 30	1	0.009	ND		
7 31	1	0.029	0.19		
8					
9 Day 3 (4-3-15)					
10 14	1	0.262	1.75		
11 18	1	0.095	0.63		
12 25	1	0.098	0.65		
13 29	1	0.209	1.40		
14 30	1	0.130	0.87		
15 31	1	0.147	0.98		
16					
17 Day 20 (4-20-15)					
18 14	1	0.035	0.23		
19 18	1	0.050	0.33		
20 25	1	0.157	1.05		
21 29	1	0.377	2.52		
22 30	1	0.224	1.50		
23 31	1	0.051	0.34		
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					

$$y = 0.1496x$$

$$R^2 = 0.9986$$

Standard Curve



Reporting limit (mg/L) = 0.10

Recovery (%) = 108.0

Precision (RPD) = 4.12

2nd source (%) = 93.5

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-2

Test Day: 0, 3, & 20

Species: *Neanthes***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst:

JB

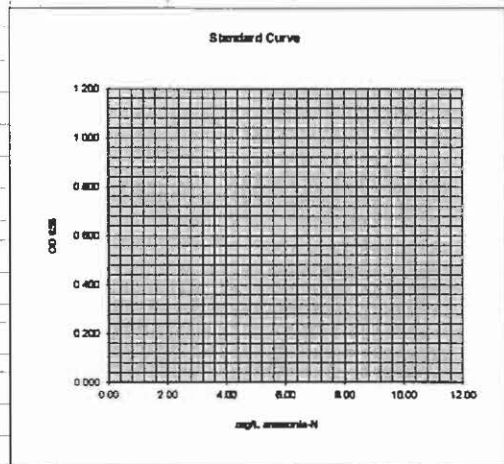
Date analysed:

4/24/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	---	---	---		
1.0 mg/L NH ₃ -N Std.	---	0.150	1.00		
3.0 mg/L NH ₃ -N Std.	---	0.485	3.00		
6.0 mg/L NH ₃ -N Std.	---	0.890	6.00		
10.0 mg/L NH ₃ -N Std.	---	1.490	10.00		
3.0 mg/L spike	---	0.495			
3.0 mg/L spike dupl.	---	0.795	0.475	05-16-15	83
5.0 mg/L 2nd source	---	0.700			



1	Day 0 (3-31-15)		
2	14	1	0.198
3	18	1	0.017
4	25	1	0.020
5	29	1	0.100
6	30	1	0.009
7	31	1	0.029

8			
9	Day 3 (4-3-15)		
10	14	1	0.262
11	18	1	0.095
12	25	1	0.098
13	29	1	0.209
14	30	1	0.130
15	31	1	0.147

16			
17	Day 20 (4-20-15)		
18	14	1	0.035
19	18	1	0.050
20	25	1	0.157
21	29	1	0.377
22	30	1	0.224
23	31	1	0.051

Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-2

Test Day: 0, 3, & 20

Species: *Neanthes***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst:

Date analysed:

JB

4/24/2015

CHAIN-OF-CUSTODY RECORDS

CUSTODY TRANSFER

Printed: 03/19/15

ARI Job No: ZV36



4611 South 134th Place, Suite 100
Tukwila WA 98168
206-695-6200 206-695-6201 (fax)

ARI Project Manager: Cheronne Oreiro	Client Contact: Dan Berlin	Sampling Event: 110603-01.02	Samples Received: 02/05/15
	Client: Anchor QEA, LLC.	Project: West Waterway Sediment Char	Sample Site: NA

LOGNUM ARI ID	CLIENT ID	MATRIX	# CONTAINERS	ANALYTICAL REQUEST	ANALYTICAL REQUEST	ANALYTICAL REQUEST	COMMENTS
15-2108 ZV36D	WWD02-0-4-150205	Sediment	1	Bioassay			NAS # 52466
15-2109 ZV36E	WWD02-4-8-150205	Sediment	1	Bioassay			NAS # 52456

TEMP: 14.5°C

PAGE 37 OF 45

Comments/Special Instructions 128326950355612155 128326950356908763 128326950356171571 128326950356999988	Relinquished By Printed Name Jennifer Millsap Company ARI Date/Time 3/19/15 1400	Received by (Signature) Printed Name GERALD IRESSARI Company NAS Date/Time 3-23-15 / 1325	Relinquished By Printed Name Company Date/Time 	Received by (Signature) Printed Name Company Date/Time
---	--	--	---	--

CUSTODY TRANSFER

Printed: 03/19/15

ARI Job No: ZV66



4611 South 134th Place, Suite 100
Tukwila WA 98168
206-695-6200 206-695-6201 (fax)

ARI Project Manager: Cheronne Oreiro	Client Contact: Dan Berlin	Sampling Event: 110103-01.02	Samples Received: 02/06/15
	Client: Anchor QEA, LLC.	Project: West Waterway Sediment Char	Sample Site: NA

LOGNUM ARI ID	CLIENT ID	MATRIX	# CONTAINERS	ANALYTICAL REQUEST	ANALYTICAL REQUEST	ANALYTICAL REQUEST	COMMENTS
15-2221 ZV66A	WWD05-0-4-150206	Sediment	1	Bioassay			NAS # 5231G
15-2224 ZV66D	WWD12-0-4-150206	Sediment	1	Bioassay			NAS # 5247G

TEMP: 14.0 °C

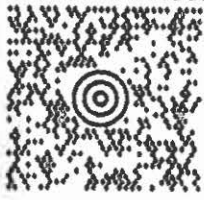
PAGE 38 OF 45

Comments/Special Instructions	Relinquished By	Received by (Signature)	Relinquished By	Received by (Signature)
	Printed Name	Printed Name	Printed Name	Printed Name
	Company	Company	Company	Company
	Date/Time	Date/Time	Date/Time	Date/Time
	<i>[Signature]</i> Jennifer Mills	<i>[Signature]</i> GERALD IRISSARRI		
	ARI	NAS		
	3/19/15 1400	3-23-15 1315		

SHIP
TO:

20/Mar/2015 03:10 9729

**NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639**



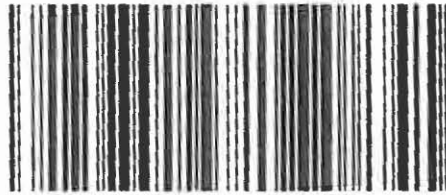
OR 973 2-01



1Z8328850358008763

UPS GROUND

TRACKING #: 1Z 832 695 03 5690 8763



SHKSHWL

LICENSE

US

9729

MAR

20

03:09:12

2015

HIP

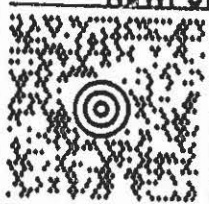
14.3.1

2P450S

SHIP
TO:

20/Mar/2015 03:37 9729

**NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639**



OR 973 2-01



1Z8326950356612156

UPS GROUND

TRACKING #: 1Z 832 695 03 5561 2155



SNK5MML LICENSE US 9729 MAR 20 09:36:19 2015 HIP 14.3.1 2P4505

SHIP
TO:

20/Mar/2015 03:43 9729

NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639



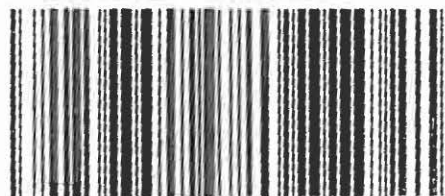
OR 973 2-01



1Z8328950356999888

UPS GROUND

TRACKING #: 1Z 832 695 03 5699 9988



SNK5HWL LICENSE US 9729 MAR 20 03:42:54 2015 MIP 14.3.1 ZP4505

SAMPLE RECEIVING
72061 606-6200
281 LABS 100
1611 S 154TH AVE
OMAHA NE 68166-3212

38 D.L.

(01)

SHIP TO:

SAMPLE RECEIVING

(541) 267-7225

WESTERN AQUATIC SCIENCES

1511 8TH AVE

10

97200 0038

TEMP: 15-5°C

MAS# 52316

373 2-01

CUSTODY SEAL

Date

5/19/05

Signature

[Handwritten Signature]

Thermo
SCIENTIFIC

90005

CUSTODY SEAL

Date

3/19/15

Signature

[Handwritten Signature]

Thermo
SCIENTIFIC

90009

CUSTC

SEA L

Date

3/1



Signature

[Handwritten Signature]

[Handwritten Mark]

Thermo
SCIENTIFIC

90009

 ANCHOR
QEA 

1 See project SAP/QAPP for analyte lists and test methods
2 email sample confirmation report to labdata@anchorqea.com

Additional notes/comments:

NITROGEN IN HEADSPACE AS PRESERVATIVE

Distribution: A copy will be made for the laboratory and client. The Project file will retain the original!



8656 3169 3053

0200

Form
C-100

FedEx Retrieval Copy

1 From
Date 11/11/01 Sender's FedEx Account Number 2115 11224
Sender's Name Amazon.com Phone 206 261 4141
Company Amazon.com
Address 410 9th Ave N Dept./Floor/Suite/Room
City Seattle State WA ZIP 98107

2 Your Internal Billing Reference www

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Recipient's Name David Lindquist Phone 206 261 4141
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Recipient's Address 3811 Yaguma Bay Road Dept./Floor/Suite/Room
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8656 3169 3053

4a Express Package Service
110 ☒ FedEx Priority Overnight
Next business morning ¹ Friday
Shipments will be delivered on Monday,
unless SATURDAY Delivery is selected.
3 ☐ FedEx 2Day
Second business day ² Thursday
Shipments will be delivered on Monday,
unless SATURDAY Delivery is selected.
20 ☐ FedEx Express Saver
Third business day
Shipments will be delivered on Monday,
unless SATURDAY Delivery is selected.
* FedEx Envelope rate not available. Minimum charge: One pound rate.
* To meet locations

4b Express Freight Service
7 ☐ FedEx 1Day Freight³
Next business day ⁴ Friday
Shipments will be delivered on Monday,
unless SATURDAY Delivery is selected.
8 ☐ FedEx 2Day Freight
Second business day ⁵ Thursday
Shipments will be delivered on Monday,
unless SATURDAY Delivery is selected.
83 ☐ FedEx 3Day Freight
Third business day ⁶ Saturday
Shipments will be delivered on Monday,
unless SATURDAY Delivery is selected.
* Call for Confirmation. ⁷ To meet locations

5 Packaging
6 ☐ FedEx Envelope⁸ 2 ☐ FedEx Pak⁹
Includes FedEx Small Pak,
FedEx Large Pak, and FedEx Sturdy Pak.
3 ☐ FedEx Box 4 ☐ FedEx Tube
1 ☒ Other
Declarative value limit \$500

6 Special Handling
3 ☐ SATURDAY Delivery
Not available for
FedEx Standard Overnight,
FedEx First Overnight, FedEx Express
Saver, or FedEx 3Day Freight.
1 ☐ HOLD Weekday
at FedEx Location
Not available for
FedEx First Overnight.
31 ☐ HOLD Saturday
at FedEx Location
Available ONLY for FedEx Priority
Overnight and FedEx 2Day
to select locations.
Include FedEx address in Section 3.
Does this shipment contain dangerous goods?
One box must be checked
☒ No 4 ☐ Yes
As per attached
Shipper's Declaration
☐ Yes
Shipper's Declaration
not required.
6 ☐ Dry Ice
Dry Ice: 9 UN 1845
Cargo Aircraft Only
Dangerous goods including dry ice cannot be shipped in FedEx packaging.

7 Payment Bill to:
110 ☒ Sender
Acct. No. in
Section 1 will
be billed.
2 ☐ Recipient 3 ☐ Third Party 4 ☐ Credit Card 5 ☐ Cash/Check
Enter FedEx Acct. No. or Credit Card No. below
Obtain Recip.
Acct. No.
Total Packages 1 Total Weight 3.6
Our liability is limited to \$100 unless you declare a higher value. See the current FedEx Service Guide for details. Credit Card Auth.

8 Residential Delivery Signature Options
If you require a signature, check Direct or Indirect:
No Signature Required
Package may be left
without obtaining a
signature for delivery. 10 ☐
Direct Signature
Someone at recipient's
address may sign for
delivery. Fee applies. 34 ☐
Indirect Signature
If no one is available at
recipient's address, someone
at a neighboring address may
sign for delivery. Fee applies. 520 ☐

Rev. Date 10/09-Part 1153281-C1184-2008 FedEx-PRINTED IN U.S.A. 3171

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3407 Client: QC Test Investigator REVIEWED PAGES 1-8 -631
 Test Type (range-finding/definitive) definitive Test Length (hr) 96
 Species *Neanthes arenaceodentata*

STUDY MANAGEMENT

Client: QC Test
 Client's Study Monitor: N/A
 Testing Laboratory: Northwestern Aquatic Sciences
 Test Location: Newport Laboratory
 Laboratory's Study Personnel:
 Proj. Man./Study Dir. G.J. IRISARRI 631
 QA Officer L.K. Nemeth
 1. Yves Natukama 2. CA Bullock 63
 3. _____ 4. _____
 5. _____ 6. _____

Study Schedule:

Test Beginning: 3-31-15 1030 Test Ending: 4-4-15 1100

TEST MATERIAL

MAILLINCKRODT (NGLIST NUMBER)
 Description: Ammonia prepared from ammonium chloride; stock prepared 12/31/13.
10,000 mg/L (=10.0 mg/mL) expressed as N (47586) 631
5-24-15

DILUTION WATER

Description: Yaquina Bay, Oregon, sea water
 Date of Preparation/Collection: 3-25-15
 Water Quality: Cond. (umhos/cm): ---- Salinity (ppt) 25.0 pH 8.0
 Hardness (mg/L as CaCO₃): ---- Alkalinity (mg/L as CaCO₃): ----
 Treatments: Filtered to ≤0.45 um, salinity adjusted with Milli-Q deionized water, aerated

TEST LOCATION

Test conducted in (circle one): room 1 room 2 trailer water bath other: _____

Randomization chart:

NORTHWESTERN AQUATIC SCIENCES
ACUTE TOXICITY TEST (ALL SPECIES)

PROTOCOL NO. NAS-XXX-_____

Test No. 999-3407 Client: _____ QC Test _____ Investigator _____

TEST ORGANISMS

Species: *Neanthes arenaceodentata* Age: 2-3 wks postemergence
Source: Aquatic Toxicology Support, Bremerton, WA Size: _____
RECEIVED: 3-27-15

Acclimation Data:

Date	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Feeding		Water changes	Comments
					amount	description		
3-27-15	17.3	7.2	28.0	7.3		TETRA MARINE	YES	
3-28-15	20.7	7.9	28.0	7.2		—	—	
3-29-15	20.5	8.0	29.0	7.5		TETRA MARINE	YES	
3-30-15	20.6	7.9	28.0	7.2		—	YES	
3-31-15	20.5	8.0	27.0	7.3		—	—	
Mean	19.9	7.8	28.0	7.3				
S.D.	1.5	0.3	0.7	0.1				
(N)	5	5	5	5				

Photoperiod during acclimation: CONSTANT LIGHT

TEST PROCEDURES AND CONDITIONS

Test concentrations (50% series recommended): 700, 441, 278, 175, 110, 0
(63% series)
Test chamber: 250 mL beakers Test volume: 100 mL
Replicates/treatment: 4 Organisms/treatment: 20 (5/repl)
Test water changes: None Aeration during test: None
Feeding: None
Duration: 24-hr, 48-hr, 96-hr Test temperature (deg.C): 20 +/- 1
Beaker placement: Stratified randomization Photoperiod: Constant light
Salinity: 28 +/- 2 ppt

MISCELLANEOUS NOTES

Test Solution Preparation:

3-31-15
651

Test Conc. (mg/L)	mL of stock (10.0 mg/mL) per 400 mL	
700	28	Bring up to 400 mL with dilution water, then split between 4 replicates
441	17.6	
278	11.1	
175	7.0	
110	4.4	
0	0	

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3407

Client _____ QC Test _____

Investigator _____

DAILY RECORD SHEET

Day 0 (3/31/15) UB

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 700	19.7	7.3	26.5	7.3	5	5	5	5
2. 441	19.8	7.4	27.5	7.3	5	5	5	5
3. 278	19.8	7.6	28.0	7.3	5	5	5	5
4. 175	19.8	7.7	28.0	7.3	5	5	5	5
5. 110	19.8	7.8	28.0	7.3	5	5	5	5
6. 0	20.0	8.1	28.0	7.4	5	5	5	5

Day 1 (4/1/15) UB

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 700	20.2	7.5	27.0	7.0	3(20)	3(20)	4(10)	4(10)
2. 441	20.2	7.6	27.5	7.1	5	5	5	5
3. 278	20.1	7.7	28.0	7.0	5	5	5	5
4. 175	19.9	7.8	28.0	7.2	5	5	5	5
5. 110	19.9	7.9	28.0	7.1	5	5	5	5
6. 0	20.1	8.1	28.0	7.1	5	5	5	5

Day 2 (4/2/15) UB

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 700	20.0	7.4	28.5	6.5	0(30)	0(30)	0(40)	0(40)
2. 441	20.1	7.7	28.5	6.1	2(30)	2(30)	0(50)	2(30)
3. 278	20.2	7.7	28.5	6.7	5	5	5	5
4. 175	20.2	7.9	28.0	7.1	5	5	5	5
5. 110	19.7	7.9	28.0	7.1	5	5	5	5
6. 0	20.0	8.1	28.0	7.1	5	5	5	5

Day 3 (4/3/15) UB

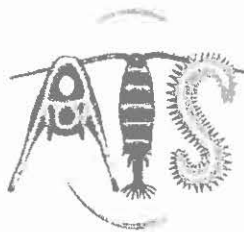
Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 700	—	—	—	—	0	0	0	0
2. 441	20.1	7.7	29.0	6.7	1(10)	0(20)	0	1(10)
3. 278	20.3	7.7	29.0	6.3	5	5	5	5
4. 175	20.3	7.7	28.5	6.3	5	5	5	5
5. 110	19.7	7.8	29.0	6.9	5	5	5	5
6. 0	20.0	8.1	27.5	7.1	5	5	5	5

Day 4 (4/4/15) UB

Conc. (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 700	—	—	—	—	0	0	0	0
2. 441	20.1	7.6	29.0	6.6	0(10)	0	0	0(10)
3. 278	20.3	7.6	29.5	6.4	3(20)	1(40)	1(40)	0(50)
4. 175	20.0	7.7	29.0	6.2	5	5	5	5
5. 110	20.1	7.7	28.5	6.8	5	5	5	5
6. 0	20.1	8.1	28.0	6.7	5	5	5	5

WQ: Mean
SD
n

(SEE PAGE 5)



Recd 3-27-15

Aquatic Toxicology Support
1849 Charleston Beach Road West
Bremerton, Washington 98312
(360) 813-1202

Order Summary

Species: <i>Neanthes arenaceodentata</i> *	Emergence Date: 9-11 March '15
Number Ordered: 390	Number Shipped: 390 + 10%
Date Shipped: 26 March '15	Salinity (ppt): 30

*Smith 1964. CSU Long Beach strain. Feed upon arrival.

Water Quality Data - test #999-3407 Neanthes QC-NH4 test

Day	Concentration	Temperature	pH	Salinity	DO
0	700	19.7	7.3	26.5	7.3
0	441	19.8	7.4	27.5	7.3
0	278	19.8	7.6	28.0	7.3
0	175	19.8	7.7	28.0	7.3
0	110	19.8	7.8	28.0	7.3
0	0	20.0	8.1	28.0	7.4
1	700	20.2	7.5	27.0	7.0
1	441	20.2	7.6	27.5	7.1
1	278	20.1	7.7	28.0	7.0
1	175	19.9	7.8	28.0	7.2
1	110	19.9	7.9	28.0	7.1
1	0	20.1	8.1	28.0	7.1
2	700	20.0	7.4	28.5	6.5
2	441	20.1	7.7	28.5	6.1
2	278	20.2	7.7	28.5	6.7
2	175	20.2	7.9	28.0	7.1
2	110	19.7	7.9	28.0	7.1
2	0	20.0	8.1	28.0	7.1
3	700				
3	441	20.1	7.7	29.0	6.7
3	278	20.3	7.7	29.0	6.3
3	175	20.3	7.7	28.5	6.5
3	110	19.7	7.8	29.0	6.9
3	0	20.0	8.1	27.5	7.1
4	700				
4	441	20.1	7.6	29.0	6.6
4	278	20.3	7.6	29.5	6.4
4	175	20.0	7.7	29.0	6.2
4	110	20.1	7.8	28.5	6.8
4	0	20.1	8.1	28.0	6.7
	MEAN	20.0	7.8	28.2	6.9
	SD	0.2	0.2	0.7	0.4
	N	28	28	28	28
	MIN	19.7	7.3	26.5	6.1
	MAX	20.3	8.1	29.5	7.4

data entry verified
against laboratory
benen sheets 5-14-15
JRF

CETIS Summary Report

Report Date: 21 Apr-15 12:12 (p 1 of 1)
 Test Code: 999-3407 16-5624-6091

Reference Toxicant 96-h Acute Survival Test						Northwestern Aquatic Sciences					
Batch ID:	11-9533-8531	Test Type:	Survival	Analyst:							
Start Date:	31 Mar-15 10:30	Protocol:		Diluent:	Yaquina Bay Seawater						
Ending Date:	04 Apr-15 11:00	Species:	Neanthes arenaceodentata	Brine:							
Duration:	4d 1h	Source:	Aquatic Toxicology Support, WA	Age:							
Sample ID:	16-1368-7806	Code:	602EEBFE	Client:	Internal Lab						
Sample Date:	31 Mar-15 10:30	Material:	Ammonia as nitrogen	Project:							
Receive Date:	31 Mar-15 10:30	Source:	Reference Toxicant								
Sample Age:	NA	Station:									
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
17-4597-6893	Proportion Survived	175	278	220.6	20.3%		Steel Many-One Rank Sum Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	mg/L	95% LCL	95% UCL	TU	Method				
08-6608-8837	Proportion Survived	EC50	241.9	218.8	267.4		Spearman-Kärber				
Proportion Survived Summary											
C-mg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	0	0	0.0%	0.0%
110		4	1	1	1	1	1	0	0	0.0%	0.0%
175		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	5.0%
278		4	0.25	0	0.6504	0	0.6	0.1258	0.2517	100.7%	75.0%
441		4	0	0	0	0	0	0	0		100.0%
700		4	0	0	0	0	0	0	0		100.0%
Proportion Survived Detail											
C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	1	1	1	1						
110		1	1	1	1						
175		1	0.8	1	1						
278		0.6	0.2	0.2	0						
441		0	0	0	0						
700		0	0	0	0						

CETIS Test Data Worksheet

Report Date:

21 Apr-15 12:11 (p 1 of 1)

Test Code:

16-5624-6091/999-3407

Reference Toxicant 96-h Acute Survival Test						Northwestern Aquatic Sciences
Start Date:	31 Mar-15 10:30	Species:	Neanthes arenaceodentata	Sample Code:	602EEBFE	
End Date:	04 Apr-15 11:00	Protocol:		Sample Source:	Reference Toxicant	
Sample Date:	31 Mar-15 10:30	Material:	Ammonia as nitrogen	Sample Station:		
C-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	10	5	5	
0	D	2	16	5	5	
0	D	3	11	5	5	
0	D	4	4	5	5	
110		1	8	5	5	
110		2	22	5	5	
110		3	24	5	5	
110		4	12	5	5	
175		1	13	5	5	
175		2	18	5	4	
175		3	21	5	5	
175		4	5	5	5	
278		1	14	5	3	
278		2	15	5	1	
278		3	3	5	1	
278		4	17	5	0	
441		1	2	5	0	
441		2	6	5	0	
441		3	19	5	0	
441		4	20	5	0	
700		1	23	5	0	
700		2	9	5	0	
700		3	1	5	0	
700		4	7	5	0	

data entry verified against laboratory bench sheets 5-14-15 JRF

Polychaete worm, *N. arenaceodentata*, acute reference toxicant test

Northwestern Aquatic Sciences

Test Type: Survival

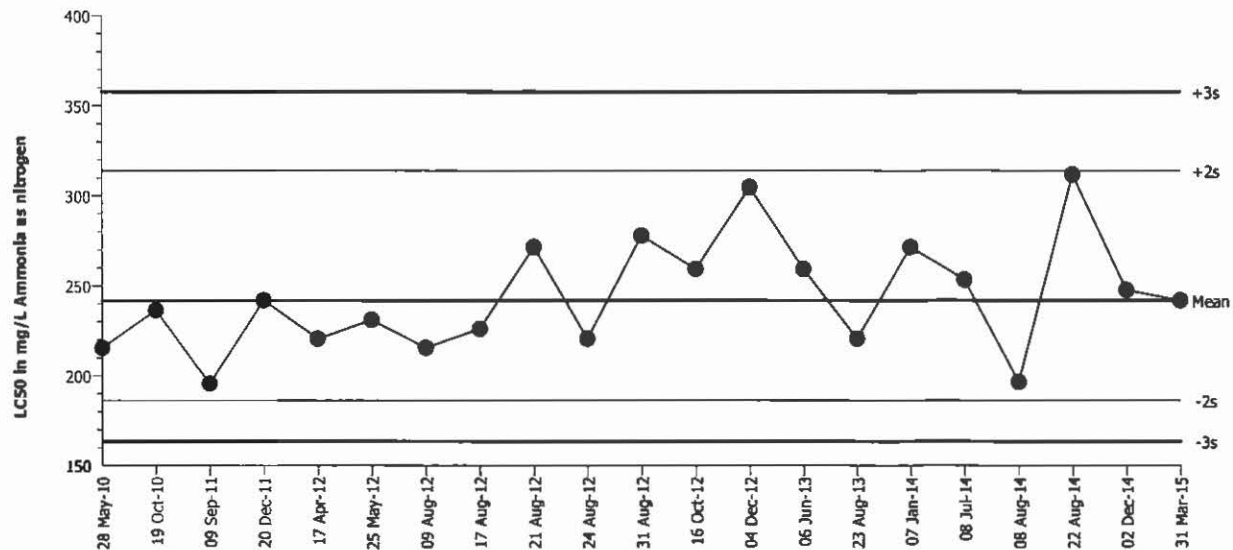
Organism: *Neanthes arenaceodentata* (Polycha)

Material: Ammonia as nitrogen

Protocol: All Protocols

Endpoint: Proportion Survived

Source: Reference Toxicant-REF

Polychaete worm, *N. arenaceodentata*, acute reference toxicant test

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2010	May	28	10:35	215.5	-26.26	-0.8804			06-3785-9046	14-2358-6368
2		Oct	19	10:25	236.4	-5.374	-0.1721			14-2393-5162	20-9902-2198
3	2011	Sep	9	13:20	195.6	-46.14	-1.621			12-6939-3159	19-5473-8057
4		Dec	20	10:10	241.9	0.1522	0.004819			18-5238-3272	11-9927-0425
5	2012	Apr	17	9:50	220.6	-21.21	-0.7029			15-1922-8238	13-4079-6866
6		May	25	9:40	231	-10.77	-0.349			02-3780-0879	06-9289-8161
7		Aug	9	8:30	215.5	-26.26	-0.8804			01-5125-7307	17-9971-3327
8			17	9:50	226	-15.78	-0.5166			19-5765-7810	12-0365-4678
9			21	11:00	271.6	29.78	0.8894			10-2483-8567	08-4339-4790
10			24	9:50	220.6	-21.21	-0.7029			07-9077-8159	10-6371-9789
11			31	9:40	277.9	36.13	1.066			15-3198-7394	09-2303-0123
12		Oct	16	11:15	259.3	17.52	0.5356			16-7030-9694	05-4630-9360
13		Dec	4	9:30	304.8	63.04	1.774			08-1804-9321	17-4513-4214
14	2013	Jun	6	9:40	259.3	17.52	0.5356			11-1706-9643	10-8401-0768
15		Aug	23	10:30	220.6	-21.21	-0.7029			08-9778-3668	05-1024-1330
16	2014	Jan	7	10:10	271.6	29.78	0.8894			19-3041-1938	17-2579-8661
17		Jul	8	11:00	253.3	11.54	0.357			00-4185-2629	10-9110-8205
18		Aug	8	10:30	196.6	-45.17	-1.584			05-2964-2150	19-0126-4454
19			22	9:50	311.9	70.14	1.95			12-9796-3055	08-9822-5275
20		Dec	2	9:25	247.5	5.772	0.1806			07-1921-6605	19-6743-2176
21	2015	Mar	31	10:30	241.9	0.1348	0.004268			16-5624-6091	08-6608-8837

SECTION D

***Mytilus galloprovincialis* sediment bioassay 865-4 data report**

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 865-4Title: *Mytilus galloprovincialis* larval sediment toxicity test of marine sediments as part of Port of Seattle – West Waterway Deepening project.Protocol: NAS-XXX-CG4/MG4, June 20, 1990. Rev. 2 (Feb.10, 1997). Based on: Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995), with modifications as specified by the Dredged Material Management Program (DMMP, formerly Puget Sound Dredged Disposal Analysis Program or PSDDA). Dredged Material Evaluation and Disposal Procedures User Manual, December 2014.

STUDY MANAGEMENT

Study Sponsor: Anchor QEA, 720 Olive Way, Suite 1900, Seattle, Washington 98101.Sponsor's Study Monitor: Ms. Cindy FieldsTesting Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, Oregon 97365.Test Location: Newport Laboratory.Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Mngr./ Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, Ph.D., Sr. Aq. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; J. B. Brown, B.S., D.V.M., Assoc. Aq. Toxicol.; Y. Nakahama, Sr.Tech.; L. Brady, Tech.Study Schedule:

Test Beginning: 4-22-15, 1320 hrs.

Test Ending: 4-24-15, 1620 hrs.

Disposition of Study Records: All raw data, reports, and other study records are stored at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Four test sediments and one reference sediments were tested. Details follow:

NAS Sample No.	5231G	5245G	5246G
Sample Description	WWD05-0-4-150206	WWD02-4-8-150205	WWD02-0-4-150205
Collection Date	2-6-15	2-5-15	2-5-15
Receipt Date	3-23-15	3-23-15	3-23-15
Interstitial Salinity (‰)	29.0	29.5	29.5

NAS Sample No.	5247G	5230G
Sample Description	WWD12-0-4-150206	WWD-REF-150319
Collection Date	2-6-15	3-19-15
Receipt Date	3-23-15	3-20-15
Interstitial Salinity (‰)	29.0	27.0

Storage: Samples were stored at 4°C in the dark.Treatments: The samples were minimally homogenized by mixing with stainless steel implements.

TEST WATER

Source: Yaquina Bay, OregonDate of Collection: 4-20-15Water Quality: Salinity 28.0 ‰, pH 8.2Pretreatment: Filtered to ≤0.45 µm, salinity-adjusted with MilliQ® deionized water, aerated.

TEST ORGANISMS

Species: *Mytilus galloprovincialis*Age: 1.0 hrs post-fertilization

Source: Mussels were purchased from Taylor Shellfish Farms, Shelton, WA and received on 4/21/15.

Acclimation: Adult animals were held in trays of flowing seawater under outside ambient conditions. Conditions the day prior to testing were: temperature, 10.7°C; pH, 7.8; salinity, 33.0 ‰; dissolved oxygen, 7.9 mg/L.

Source of Gametes: 3 females, 3 males

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 1 L covered borosilicate glass beakers

Test Volumes: 18 g of test or reference sediment with 900 ml of test water added. Sediment was mixed for 10 seconds and allowed to settle for the normal period of 4 hours as specified in the protocol.

Replicates/Treatment: 5 (plus a 6th water quality replicate).

Sediment Salinity Adjustment: None required.

Initial Concentration of Test Organisms: 25.7/ml

Water volume changes per 24 hours: None

Volume of Subsamples Taken for Counting: 10 ml

Aeration: Yes

Feeding: None

Acceptance Criteria: The percent normal larvae in the seawater control must be $\geq 70\%$ at the end of the test.

Performance Criteria: For DMMP projects, the mean seawater-normalized combined mortality and abnormality in the reference sediments must be $\leq 35\%$.

Effects Criteria: The effects criteria used were: 1) mortality; 2) abnormal development to the fully-shelled stage; and 3) the combined mortality/abnormality endpoint. Normal development is defined as transformation to the fully shelled, straight-hinged, D-shaped prodissoconch I stage. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed, and 3) the number of normal larvae observed. The results were expressed as: 1) percent abnormality; 2) percent mortality; 3) combined percent mortality and abnormality; and 4) normalized (to the seawater control) a) percent mortality and b) combined percent mortality and abnormality.

Water Quality and Other Test Conditions: The temperature, pH, salinity, and dissolved oxygen were measured in the water quality replicate test chamber daily. Total soluble sulfide and total ammonia-N were measured in the overlying water of the water quality replicate test chamber on days 0 and 2. Total soluble sulfide and total ammonia-N were measured using Hach reagents based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. The photoperiod was 14:10, L:D.

DATA ANALYSIS METHODS

All three standard endpoints, percent abnormal, percent combined mortality/abnormality, and percent mortality have occasionally been computed both with, and without, normalization for the seawater control. Endpoints in this report have been computed according to the following formulas:

$$\text{PABN (Percent Abnormality)} = 100 \cdot (A/T)$$

$$\text{PABND (Combined Percent Mortality/Abnormality)} = 100 \cdot ((I-N)/I)$$

$$\text{PMORT (Percent Mortality)} = 100 \cdot ((I-T)/I)$$

$$\text{NPM (Normalized Percent Mortality)} = 100 \cdot (1 - (T/TS))$$

$$\text{NCMA (Normalized Combined Percent Mortality/Abnormality)} = 100 \cdot (1 - (N/NS))$$

where the following are counts per 10 ml subsample:

N = normal larvae counted

A = abnormal larvae counted

T = N+A (total larvae counted)

I = number of inoculated embryos (from average of zero time counts)

TS = average of total larvae counted in seawater controls

NS = average of normal larvae counted in seawater controls

Control and treatment means and standard deviations for the biological endpoints described above and for water quality data were computed using Microsoft EXCEL 2010. The software used for statistical comparisons was BioStat (version Feb 9, 2006 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. The number normal in each test sediment was compared against that in the appropriate reference sediment. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.10 level of significance.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The reference toxicant test is a standard multi-concentration toxicity test using copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix II. The reference toxicant test is conducted following EPA/600/R-95/136 (Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, August 1995).

Test No.: 999-3413

Reference Toxicant and Source: Copper as copper sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Argent Lot #0195, 1.0 mg/ml stock prepared 6-19-14.

Test Date: 4-22-15

Dilution Water Used: Yaquina Bay, Oregon, seawater at 30.0‰; pH, 8.1.

Result: 48-hr EC50, 8.59 $\mu\text{g/L}$ Cu. This result is within the laboratory's control chart warning limits (8.07 to 13.5 $\mu\text{g/L}$ Cu).

RESULTS AND DISCUSSION

Observations of water quality parameters during the test are summarized in Table 1. Individual water quality measurements are located in the raw data (Appendix II).

The measurements of standard water quality parameters were all within protocol specified ranges (Table 1). Sulfides were not detected in the overlying bioassay water (detection limit 0.02 mg/L). Total ammonia-N ranged from <0.1 mg/L to 0.3 mg/L.

Means and standard deviations of the number normal and the normalized combined percent mortality and abnormality (NCMA) endpoints for sediments are summarized in Table 2. Detailed data organized by sample and replicate, including the larval counts, for all calculated endpoints are given in Appendix II. A total of five replicate subsamples were recounted (QC counts) as a check on the acceptability of the initial counts (Appendix II). In all instances the QC counts were close (coefficients of variation from 2 to 4 for counts of normal larvae) to the initial counts and were considered acceptable.

The test met the control acceptance criterion of $\geq 70\%$ normal in the seawater control; the control mean percent normality was 75.8%. The percent normal of the reference sediment, WWD-REF-150319 was 89.8% of the seawater control, meeting the DMMP reference sediment performance standard of criterion $\geq 65\%$.

The test control acceptance criterion was met, the reference sediment performance criterion was met and positive control performance was within the laboratory's acceptance limits, it is concluded that the test has developed acceptable data for use in making management decisions.

The number normal was used for data analysis and interpretation rather than the NCMA due to the NCMA resulting in negative numbers in some replicates. None of the test sediments was statistically significantly lower than the reference sediment WWD-REF-150319 for number of normal larvae.

Interpretation was based on guidelines from the "Dredged Material Evaluation and Disposal Procedures User Manual, December 2014. For a test sediment to fail under these guidelines, under the single hit rule, the mean NCMA must be >20% (number normal <80% of control), and 15% (dispersive) or 30% (nondispersive) absolute over the mean reference sediment NCMA (absolute below the mean reference number normal), and statistically different from the reference ($\alpha = 0.10$).

No test sediment failed the single-hit rule for dispersive sites or nondispersive sites (Table 3).

STUDY APPROVAL

 6/3/15
Project Manager/ Study Director Date

 6-3-15
Quality Assurance Unit Date

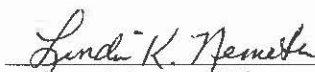
 6/2/15
Assistant Laboratory Director Date

Table 1. Summary of water quality conditions during the tests of mussel, *Mytilus galloprovincialis*, larvae exposed to marine sediments.

Parameter	Mean \pm SD	Minimum	Maximum	N
Temperature ($^{\circ}$ C)	15.2 \pm 0.1	15.0	15.4	18
pH	8.1 \pm 0.0	8.0	8.2	18
Salinity (‰)	28.4 \pm 0.4	28.0	29.0	18
Dissolved Oxygen (mg/L)	7.8 \pm 0.2	7.3	8.1	18
Dissolved Sulfide (mg/L)	---	<0.02	<0.02	12
Total Ammonia-N (mg/L)	---	<0.1	0.3	12

Table 2. Means and standard deviations (n=5) of number normal larvae and NCMA (combined mortality and abnormality, normalized to the seawater control) of mussel, *Mytilus galloprovincialis*, larvae exposed to marine sediments.

Sample Description	Number normal (mean \pm SD)	Normalized combined percent mortality & abnormality (NCMA)
Seawater control	195 \pm 13	0.0 \pm 6.5
WWD02-0-4-150205 (NAS# 5246G)	161 \pm 11	17.3 \pm 5.6
WWD02-4-8-150205 (NAS# 5245G)	166 \pm 12	14.9 \pm 6.2
WWD05-0-4-150206 (NAS# 5231G)	151 \pm 30	22.4 \pm 15.6
WWD12-0-4-150206 (NAS# 5247G)	196 \pm 12	-0.5 \pm 6.2
WWD-REF-150319 (NAS# 5230G)	175 \pm 45	10.2 \pm 23.2

*Percent mortality was significantly higher than that in reference sediment ($p < 0.10$).

Table 3. Single-hit criteria interpretation of *Mytilus* larval sediment bioassay data. The number normal was used for data interpretation rather than the NCMA due to the NCMA resulting in negative numbers in some replicates.

Sample description	Number normal (mean \pm SD)	Significantly different from the reference at $\alpha = 0.10$?	Difference from the reference (proportion)	Failure under 1-hit dispersive rule? (Greater than 0.15)	Failure under 1-hit nondispersive rule? (Greater than 0.30)
Seawater control	195 \pm 13	---	---	---	---
WWD02-0-4-150205 (NAS# 5246G)	161 \pm 11	No	0.07	No	No
WWD02-4-8-150205 (NAS# 5245G)	166 \pm 12	No	0.05	No	No
WWD05-0-4-150206 (NAS# 5231G)	151 \pm 30	No	0.12	No	No
WWD12-0-4-150206 (NAS# 5247G)	196 \pm 12	No	-0.11	No	No
WWD-REF-150319 (NAS# 5230G)	175 \pm 45	---	---	---	---
If the mean NCMA for a test sediment is >20% (<80% number normal compared to control), and is 15% (dispersive) or 30% (nondispersive) absolute over the mean reference sediment NCMA (absolute below the mean reference number normal), and statistically different from reference ($\alpha = 0.10$), it fails under the single-hit rule.					

APPENDIX I
PROTOCOL

TEST PROTOCOL

BIVALVE LARVAL SEDIMENT BIOASSAY

1. INTRODUCTION:

- 1.1 Purpose of Study: The purpose of this study is to identify test sediments that are toxic to bivalve larvae.
- 1.2 Summary of Method: The 48- to 60-hr static test is performed using newly fertilized embryos of the Pacific oyster, *Crassostrea gigas* or blue mussel, *Mytilus* sp. Eighteen grams of test sediment suspended in 900 mL of test water are vigorously mixed for 10 sec in the 1 L glass test vessels to produce the test medium. After 4 hr, the test containers are inoculated with <2-hr-old embryos at a level sufficient to yield a final concentration of 20-40 per ml. Five replicates are used for each treatment, reference, and control sediment (A sixth replicate is used only for water quality measurements). Mortality, abnormal development, and combined mortality/abnormality during exposure are the response criteria used. The mean \pm S.D. for each treatment and test endpoint is given in the final report. Between-treatment comparisons may be made with Student's t-test, or by using an analysis of variance with an appropriate post-hoc test.

2. STUDY MANAGEMENT:

2.1 Sponsor's Name and Address:

2.2 Sponsor's Study Monitor:

2.3 Name of Testing Laboratory:

Northwestern Aquatic Sciences
Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

2.4 Test Location: _____

2.5 Laboratory's Personnel to be Assigned to the Study:

Project Manager/Technical Director: _____
Qual. Assurance Officer: _____
Aquatic Biologist: _____
Aquatic Biologist: _____

2.6 Proposed Testing Schedule: Tests to begin within 2 weeks (8 weeks if held under nitrogen for PSDDA) of sample collection. Reference toxicant test to be run concurrently.

2.7 Good Laboratory Practices: Tests are conducted according to Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are marine sediments. The collected sediments are placed in solvent and acid cleaned 1 L glass jars fitted with TFE-lined screw caps. Samples are stored at 4°C in the dark for up to 14 days. For PSDDA testing, the samples may be stored under nitrogen at 4°C in the dark in the original sealed containers for up to 8 weeks prior to testing. In addition to the test sediments, one or more reference sediments (a clean sediment with physical characteristics similar to the test sediments) must be employed. A second control sediment from a clean site may also be used (optional).

4. TEST WATER

Test water is filtered Yaquina Bay seawater adjusted to a salinity of 28 ppt. The water is pumped daily from Yaquina Bay into a 6000 gal seasoned fiberglass reservoir from which it is supplied under pump pressure to the laboratory. Filtration is accomplished using a sand filter followed by a medium porosity (10-25 μ m) cartridge filter, then a ≤ 0.45 μ m filter. An alternative seawater supply of similar quality may be used. The use of sterile seawater may be desirable.

5. TEST ORGANISMS

- 5.1 Species: Pacific oyster, *Crassostrea gigas* or blue mussel *Mytilus* sp.
- 5.2 Source: Adult oysters are purchased from Oregon Oyster Co., Newport, OR, a commercial grower. Blue mussels are purchased from a commercial supplier such as Carlsbad Aquafarms, Carlsbad, CA.
- 5.3 Age at Study Initiation: Less than two-hour-old embryos.
- 5.4 Conditioning/Acclimation of Adults: Adult bivalves are conditioned/acclimated by holding for up to eight weeks in seasoned fiberglass water trays supplied with a minimum of 1 L/min of unfiltered Yaquina Bay, OR water (22-32 ppt) at a temperature of approximately 18-22°C (14-18°C for mussels). Supplemental feeding with cultured algae may be desirable. A 14 hour/10 hour light-dark photoperiod is employed.
- 5.5 Spawning and Fertilization: Adult bivalves are cleaned by brushing and placed into spawning trays supplied with seawater. The bivalves are spawned by gradually increasing the water temperature to 5-10°C above the conditioning temperature over approximately a one hour period. Sperm from a sacrificed male may be added to the spawning tray to aid stimulation of natural spawning. If spawning does not occur, the water is cooled and the cycle is repeated. Bivalves that begin spawning are rinsed and isolated in clean sterile seawater for collection of gametes.

Eggs from two or more females are fertilized by addition of sperm from two or more males at a concentration of 10^5 to 10^7 /ml. After fertilization, the embryo density is adjusted to approximately 2000-4000/ml by dilution with 28 ppt seawater. Embryos are kept suspended by frequent gentle agitation with a perforated plunger and the temperature is maintained at test temperature. The quality of the embryos is verified before testing by microscopic examination. Less than 2-hr-old embryos are used in the test.

6. DESCRIPTION OF TEST SYSTEM

- 6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are covered 1 L glass beakers. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or placement in a temperature-controlled environmental chamber. Aeration is generally recommended but not required by PSEP unless dissolved oxygen falls below 60% saturation. Aeration is required for PSDDA. The test is performed under a 14:10 L:D photoperiod with illumination supplied by fluorescent lamps.
- 6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027. (NAS SOP No. G-1.) New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The experimental design consists of exposure of bivalve embryos to a number of test sediments, one or more reference sediments, a clean sand control sediment (optional), and a no-sediment (seawater) control. Each treatment consists of five replicate test containers, each containing from 20,000 to 40,000 bivalve embryos. A sixth replicate series is used for water quality measurements. Complete randomization of test containers is used.

7.2 Test Procedure: Eighteen grams of test or control sediment is added to the appropriate 1 L test container and the test water is added to a final volume of 900 mL. The containers are vigorously mixed for 10 seconds and then allowed to settle for 4 hr. Each test vessel is then inoculated with 20,000 to 40,000 <2-hr-old bivalve embryos (~ 1 ml of embryo stock). The embryos are incubated at $20 \pm 1^\circ\text{C}$ for oysters ($16 \pm 1^\circ\text{C}$ for mussels) for 48-60 h to permit development into normal prodissoconch I stage larvae. Immediately after inoculation of the test vessels with embryos, 10 ml aliquots are removed from each of the five no-sediment control replicates and preserved in 5% buffered formalin. These are counted at a later date to establish the average initial embryo concentration.

The test is terminated after 48-60 hr by carefully decanting the supernatant liquid into a 1000 ml beaker. Ten milliliter aliquots are then taken from each beaker, placed into a 30 ml glass vial, and preserved in 5% buffered formalin. The beaker contents are gently agitated using a perforated plastic plunger at the time of aliquot removal to ensure that the aliquots accurately reflect the embryo/larval concentration of the entire test vessel. Larvae are subsequently counted in the 30 ml vials using an inverted compound microscope to determine the total number of larvae and the number failing to develop a complete shell; these data are used for calculating the percent mortality, percent abnormality, and percent combined mortality and abnormality (percent abnormal/dead). Approximately 10% of the samples may be counted by a second investigator as a QA/QC measure.

7.3 Effect Criteria: Effect criteria are: 1) mortality of the embryos, and 2) abnormal development (failure to produce completely developed shells). A third effect expression is a combination of these two, percent abnormal/dead. This is functionally equivalent to percent normal.

The initial number of embryos are determined by averaging the counts of all eggs in initial subsamples of the five seawater control test containers. Live abnormal larvae are those observed at 48-60 hr in which a fully shelled, straight-hinged, D-shaped prodissoconch I stage has been reached.

7.4 Test Conditions: The test exposure duration is 48-60 h. The test temperature is $20 \pm 1^\circ\text{C}$ for oysters and $16 \pm 1^\circ\text{C}$ for mussels. The salinity is 28 ± 1 ppt. The photoperiod is a 14:10 hr, L:D cycle of fluorescent light. Aeration is not required for PSEP unless dissolved oxygen falls below 60% saturation; aeration is required for PSDDA (dissolved oxygen must be maintained at ≥ 4 mg/L). Test chambers are 1 L glass beakers held in a controlled temperature environment.

7.5 Feeding: Embryos are not fed during the test.

7.6 Test Duration, Type and Frequency of Observations, and Methods: The test duration is 48-60 hours. The type and frequency of observations to be made during the test are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION
<u>Biological Data</u>	
Mortality and abnormal development	End of test (at 48-60 hours)
<u>Physical and Chemical Data</u>	
Temperature, dissolved oxygen, salinity, & pH	Daily in water quality beakers
Ammonia-N and sulfide	Beginning & end of test in water quality beakers

Temperature is measured with a calibrated mercury thermometer or telethermometer. Dissolved oxygen is directly measured in test vessels using a polarographic oxygen probe calibrated according to the

manufacturer's recommendations. Salinity is measured using a refractometer. The pH is measured with a pH probe and a calibrated meter with scale divisions of 0.1 pH units. Ammonia-N is measured using the HACH Model FF-3 test kit (ammonia-N detection limit 0.1 mg/L). Sulfide is measured using the HACH Hydrogen Sulfide Test Kit Model HS-WR (sulfide detection limit 0.01 mg/L).

- 7.7 Criteria of Test Acceptance: The test results are acceptable if at least 70% of the seawater control larvae achieve a normal, D-shaped prodissoconch I stage. Also, for PSDDA, effects in the reference sediments must be $\leq 35\%$ over the seawater control.

8. DATA ANALYSIS METHODS

Prior to issuance of the July 1995 revision of the PSEP recommended guidelines for conducting laboratory bioassays, no specific guidance was given for the computation of endpoints. All three standard endpoints, percent abnormal, percent combined mortality/abnormality, and percent mortality have occasionally been computed both with, and without, normalization for the seawater control. In order to be consistent with what we assume to be the PSAMP format, all endpoints given in reports are usually the non-normalized endpoints. In addition, normalized percent mortality (NPM), and normalized combined mortality/abnormality (NCMA) are computed and are included in the raw data computer printouts. The formulas employed for each of these computations are as follows:

$$\text{PABN (Percent Abnormality)} = 100 \cdot (A/T)$$

$$\text{PABND (Combined Percent Mortality/Abnormality)} = 100 \cdot ((I-N)/I)$$

$$\text{PMORT (Percent Mortality)} = 100 \cdot ((I-T)/I)$$

$$\text{NPM (Normalized Percent Mortality)} = 100 \cdot (1 - (T/TS))$$

$$\text{NCMA (Normalized Combined Percent Mortality/Abnormality)} = 100 \cdot (1 - (N/NS))$$

where the following are counts per 10 ml subsample:

N = normal larvae counted

A = abnormal larvae counted

T = N+A (total larvae counted)

I = number of inoculated embryos (from average of zero time counts)

TS = average of total larvae counted in seawater controls

NS = average of normal larvae counted in seawater controls

Other endpoints may be computed at client request.

The means and standard deviation are then calculated for each treatment level. Between-treatment comparisons for each biological endpoint may be made using a t-test or a one-way analysis of variance F test (Snedecor and Cochran 1967). An arcsine square root data transformation is required for proportional data.

9. REPORTING

A standard NAS report of the test results must include the following information: name and identification of the test; the investigator and laboratory; sediment holding temperature data; information on the test sediment including the interstitial salinity; information on the source of seawater used; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including water quality; information about any aeration that may have been required; definition of the effect criteria and other observations; unusual responses, if any, in the control treatment; individual replicate and mean and S.D. data for larval mortality after 48-60 hr; individual replicate and mean and S.D. data for larval abnormalities after 48-60 hr; individual replicate and mean and S.D. data for combined abnormality and mortality; 48-hr LC50 and EC50 with reference toxicant; a description of data analysis methods and documentation of statistical test results; any unusual information about the test or deviations from procedures.

10. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect, and the dated signatures of

the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

11. REFERENCE TOXICANT

Reference toxicant testing should be included with each study or at regular intervals as defined in the Quality Assurance Program of the laboratory.

12. REFERENCED GUIDELINES

ASTM. 1989. Standard guide for conducting static acute toxicity tests with embryos of four species of saltwater bivalve molluscs. ASTM Standard Method No. E 724-89. Am. Soc. Test. Mat., Philadelphia, PA.

PSDDA. 1990. Summary and conclusions of the Puget Sound Dredged Disposal Analysis (PSDDA) Bioassay Workshop, memorandum for the record. U.S. Army Corps of Engineers, Seattle District.

Puget Sound Estuary Program. 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound sediments. Prepared for U.S. Env. Prot. Agency, Region 10, Office of Puget Sound, Seattle, WA and Puget Sound Water Quality Authority, P.O. Box 40900, Olympia, WA.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods. Sixth ed., The Iowa State Univ. Press. Ames, Iowa., 593 pp.

Weber, C.I. (Ed.) 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027.

13. APPROVALS

_____	_____	for _____
Name	Date	
_____	_____	for NORTHWESTERN AQUATIC SCIENCES
Name	Date	

APPENDIX II

RAW DATA

**TEST DESCRIPTION, MONITORING, AND RESULTS
BENCHSHEETS**

SEDIMENT LARVAL TEST BASED ON PSEP PROTOCOLS

Test No. 865-4 Client Anchor QEA Investigator V. J.

STUDY MANAGEMENT

Client: ANCHOR QEA, LLC, 720 Olive Way, Suite 1900, Seattle, WA 98101

Client's Study Monitor: Ms. Cindy Fields

Test Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Man./Study Dir. G. J. RISSARRI 65

QA Officer, L.K. Nemeth

1. LES Nucleation —

2. Lauren Brady LB

3. J. Brown 123

4. R.S Caldwell ASD

5. GABLER 003

6

Study Schedule:

Test Beginning: 4-22-15 1320

Test Ending: 4-24-15 1620

TEST MATERIAL

General description (see sample logbook/chain-of-custody for details):

NAS Sample No.:	5231G	5245G	5246G	5247G	5230G
Description:	WWD05-0-4-150206	WWD02-4-8-150205	WWD02-0-4-150205	WWD12-0-4-150206	WWD-REF-150319
Collection Date:	2/6/2015	2/5/2015	2/5/2015	2/6/2015	3/19/2015
Receipt Date:	3/23/2015	3/23/2015	3/23/2015	3/23/2015	3/20/2015
Inters.Salinity (ppt):	29.0	29.5	29.5	29.0	27.0
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
Inters.Salinity (ppt):					

Error codes: 1) correction of handwriting error

2) written in wrong location; entry deleted

3) wrong date deleted, replaced with correct date

4) error found in measurement: measurement repeated

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Test No. 865-4 Client Anchor QEA Investigator

[illegible]

Test No.	865-4	Client	Anchor QEA	Investigator
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Species: Mytilus galloprovincialis Date Received: 4-21-15
Source: TAYLOR SHELLFISH FARMS, SHELTON, WA

Acclimation Data:					
Date	Temp. (deg.C)	pH	Sal (ppt)	DO (mg/L)	Comments
4-22-15	10.7	7.8	33.0	7.9	HELD OUTSIDE IN TRAYS OF FLOWING SEAWATER
Mean					
S.D.					
(N)					

Source: Yaquina Bay, Oregon, sea water
Date of Collection: 4-20-15 Salinity (ppt) 28.0 pH 8.2
Treatments: filtered to 0.4 um, salinity-adjusted with MilliQ deionized water, aerated
0.45 6.1
5-29-15

Spawning: Initial: 0920 AM/PM Final: 1130 AM/PM Fertilization: 1220 AM/PM
Number of organisms used: females: 3 males: 3
Egg Dilution (1 ml diluted to 500 ml):
Count/ml of dilution: 1. 49 2. 45 3. 49 Mean: 47.7
Dilution factor = DF (mean x 500/25,000) = 0.95

Test chambers: 1 L glass beakers covered with watchglasses
 Test volumes: 18 g of test sediment; 900 ml of test water
 Replicates/treatment: (5) 5 Organisms/treatment: (20-40/ml) 25.7
 Use 6th replicate/test sediment for water quality and development assessment beaker
 Test water changes: None Aeration: yes Feeding: none Photoperiod: 14L:10D
 Test temperature (deg.C): mussels 16, oysters 20, echinoderms 15
 Beaker placement: Total randomization
 Larval stock (20,000-40,000/ml) inoculation volume: 1.0 ml; <2-hr old
 Subsample size for counting: 10 ml

Page 3 of 28

SEDIMENT LARVAL TEST BASED ON PSEP PROTOCOLS

Test No. 865-4 Client _____ Anchor QEA _____ Investigator _____Test conducted in (circle one): room 1 room 2 trailer water bath other: Room # 4Randomization chart: TOP SHELF

3									36
2									35
1									34

Randomization chart: FRONT

Randomization chart:

Randomization chart:

Randomization chart:

SEDIMENT LARVAL TEST BASED ON PSEP PROTOCOLS

Test No. 865-4 Client Anchor QEA Investigator

WATER QUALITY RECORD

Day 0 (4/22/15) ✓overlying NH₃ sampled (days 0 and 2)? YES

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	S (mg/L)	Comments
10	15.0	8.0	28.0	8.1	<0.02	
14	15.1	8.0	28.0	7.7	<0.02	
17	15.0	8.0	29.0	7.7	<0.02	
23	15.1	8.1	28.0	7.5	<0.02	
28	15.3	8.1	29.0	7.3	<0.02	
34	15.3	8.1	29.0	7.3	<0.02	

Day 1 (4/23/15) ✓

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Comments
10	15.1	8.1	28.0	7.9	
14	15.1	8.1	28.0	7.9	
17	15.0	8.1	28.5	7.9	
23	15.1	8.1	28.5	7.7	
28	15.3	8.1	28.0	7.9	
34	15.4	8.1	29.0	7.9	

Day 2 (4/24/15) ✓ / inoverlying NH₃ sampled (days 0 and 2)? YES

Beaker No.	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	S (mg/L)	Comments
10	15.1	8.2	28.0	8.0	<0.02	
14	15.1	8.1	29.5 28.0	8.0	<0.02	④ ✓ 4/24/15
17	15.1	8.1	28.0	8.0	<0.02	
23	15.2	8.1	29.0	8.0	<0.02	
28	15.4	8.1	28.5	8.1	<0.02	
34	15.4	8.1	28.0	8.0	<0.02	

Test No. 865-4 Client Anchor QEA Investigator _____

Re 4/27/15

[illegible]

Test No. 865-4 Client Anchor QEA Investigator

601 4-30-15

[illegible]



TAYLOR SHELLFISH FARMS

SE 130 LYNCH RD, SHELTON, WA 98584
PH: (360) 426-6178 FAX: (360) 426-3643
WASHINGTON CERTIFICATION #: WA 46SP



10.55 UNPROCESSED MUSSELS

Original Harvesters Cert#: WA 46 SP
Original Harvest Date: 4/19/2015
Final Harvest Date: 4/19/2015
Harvested In: Washington State
Harvest Area: Totten Inlet
Wet Stored:

Airbill #

SHIP TO:

NW AQUATIC

Cert #	
PO #	
ITEM #	

161

Rec'd 4-21-15

Perishable Keep Refrigerated 2-5 C°
Farm Raised Shellfish - Product of USA

Taylor Shellfish Farms
Shelton, WA 98584
360-426-6178

WET STORED:
UNPROCESSED MUSSELS

SHIP TO: NW AQUATIC
HARVEST DATE: 4/19/2015
HARVESTED IN: Washington State
HARVEST AREA: Totten Inlet
QUANTITY & TYPE: 10.55

TEST DATA ANALYSIS RECORDS

Endpoints Data Entry and Calculations File

BKR=beaker number

INIT=number of inoculated embryos (from average of zero-time counts)

NORM=number normal

ABN=number abnormal

TOTAL=NORM+ABN

PMORT=percent mortality=100((INIT-TOTAL)/INIT)

PABN=percent abnormality=100(ABN/TOTAL)

PABND=combined percent mortality and abnormality=100((INIT-NORM)/INIT)

NPM=normalized percent mortality=100(1-(TOTAL/TS))

where TS=average of total larvae counted in seawater controls

NCMA=normalized combined percent mortality and abnormality=100(1-(NORM/NS))

where NS=average of normal larvae counted in seawater controls

zero counts	
a	278
b	255
c	238
d	255
e	282
Mean =	257

NS (mean normal)	TS (mean total)
195.0	214.2

%normal in SW cont
relative to INIT

75.8

% normal in reference sed
relative to SW cont

69.6

INDEX	NAS BKR SMPL	CLIENT DESCRIP	REPL	INIT	NORM	ABN	TOTAL	PMORT	PABN	PABND	NPM	NCMA	NORM	PMORT	PABN	PABND	NPM	NCMA
1	18 swcontrol	swcontrol	1	257	193	17	210	18.4	8.1	25.0	2.0	1.0						
2	19 swcontrol	swcontrol	2	257	217	28	245	4.7	11.4	15.6	-14.4	-11.3	Mean	195	18.7	8.9	24.2	0.0
3	5 swcontrol	swcontrol	3	257	185	13	198	23.0	6.6	28.1	7.6	5.1	SD	13	7.0	1.9	4.9	6.4
4	5 swcontrol	swcontrol	4	257	190	21	211	18.0	10.0	26.1	1.5	2.8	n	5	5	5	5	5
5	36 swcontrol	swcontrol	5	257	190	17	207	19.5	8.2	26.1	3.4	2.8						
6	10 swcontrol	swcontrol	6 wq replicate	257														
7	21 5248G	WWD02-0-4-150205	1	257	171	5	176	31.6	2.8	33.5	17.8	12.3	Mean	161	35.1	3.5	37.3	22.0
8	28 5248G	WWD02-0-4-150205	2	257	184	4	188	34.7	2.4	36.2	21.8	15.9	SD	11	3.2	2.0	4.2	3.9
9	33 5248G	WWD02-0-4-150205	3	257	144	11	155	39.7	7.1	44.0	27.6	26.2	n	5	5	5	5	5
10	32 5248G	WWD02-0-4-150205	4	257	158	5	163	36.6	3.1	38.6	23.9	19.0						
11	31 5248G	WWD02-0-4-150205	5	257	169	4	173	32.7	2.3	34.3	19.2	13.3						
12	34 5248G	WWD02-0-4-150205	6 wq replicate	257														
13	18 5245G	WWD02-4-8-150205	1	257	183	4	187	27.3	2.1	28.8	12.7	6.2	Mean	168	34.3	1.8	35.5	21.1
14	9 5245G	WWD02-4-8-150205	2	257	160	4	164	36.2	2.4	37.8	23.4	17.9	SD	12	4.7	1.0	4.7	5.6
15	30 5245G	WWD02-4-8-150205	3	257	168	3	171	33.5	1.8	34.7	20.2	13.8	n	5	5	5	5	5
16	12 5245G	WWD02-4-8-150205	4	257	150	4	154	40.1	2.8	41.7	28.1	23.1						
17	27 5245G	WWD02-4-8-150205	5	257	169	0	169	34.3	0.0	34.3	21.1	13.3						
18	23 5245G	WWD02-4-8-150205	6 wq replicate	257														
19	35 5231G	WWD05-0-4-150206	1	257	158	7	165	35.8	4.2	38.8	23.0	19.0	Mean	151	37.8	6.2	41.1	25.1
20	24 5231G	WWD05-0-4-150206	2	257	171	6	177	31.2	3.4	33.5	17.4	12.3	SD	30	10.0	5.3	11.8	12.0
21	6 5231G	WWD05-0-4-150206	3	257	188	10	198	30.4	5.6	34.3	16.4	13.3	n	5	5	5	5	5
22	15 5231G	WWD05-0-4-150206	4	257	98	18	116	54.9	15.5	61.9	45.8	49.7						
23	20 5231G	WWD05-0-4-150206	5	257	181	4	185	35.8	2.4	37.4	23.0	17.4						
24	28 5231G	WWD05-0-4-150206	6 wq replicate	257														
25	11 5247G	WWD12-0-4-150208	1	257	213	6	219	14.9	2.7	17.2	-2.2	-9.2	Mean	198	22.2	2.1	23.8	6.5
26	25 5247G	WWD12-0-4-150208	2	257	181	4	185	28.1	2.2	29.6	13.6	7.2	SD	12	4.9	0.8	4.7	5.9
27	28 5247G	WWD12-0-4-150208	3	257	202	2	204	20.7	1.0	21.5	4.8	-3.6	n	5	5	5	5	5
28	3 5247G	WWD12-0-4-150208	4	257	192	3	195	24.2	1.5	25.3	9.0	1.5						
29	13 5247G	WWD12-0-4-150208	5	257	192	6	198	23.0	3.0	25.3	7.6	1.5						
30	17 5247G	WWD12-0-4-150208	6 wq replicate	257														
31	2 5230G	WWD-REF-150319	1	257	154	2	156	39.3	1.3	40.1	27.2	21.0	Mean	175	30.9	1.5	31.9	17.0
32	7 5230G	WWD-REF-150319	2	257	202	2	204	20.7	1.0	21.5	4.8	-3.6	SD	45	17.7	0.8	17.6	21.2
33	4 5230G	WWD-REF-150319	3	257	234	4	238	7.5	1.7	9.0	-11.1	-20.0	n	5	5	5	5	5
34	1 5230G	WWD-REF-150319	4	257	170	2	172	33.1	1.2	33.9	19.7	12.8						
35	22 5230G	WWD-REF-150319	5	257	116	3	119	53.7	2.5	54.9	44.4	40.5						
36	14 5230G	WWD-REF-150319	6 wq replicate	257														

Project Name: P865-4 Mytilus Number Normal

Sample: x1
 Samp ID: WWD02-0-4-150205
 Alias: NAS# 5246G
 Replicates: 5
 Mean: 161.2
 SD: 10.849
 Tr Mean: 161.2
 Trans SD: 10.849

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 175.2
 SD: 45.158
 Tr Mean: 175.2
 Trans SD: 45.158

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 21.309 SS: 8627.6 K: 5 b: 91.128 Alpha Level: 0.05 Calculated Value: 0.9625 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 8.16 Test Residual SD: 5.871 Ref. Residual Mean: 34.24 Ref. Residual SD: 23.953 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 2.3647 Critical Value: ≥ 1.860 Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$ Degrees of Freedom: 4 Experimental Alpha Level: 0.1 Calculated Value: 0.6741 Critical Value: ≥ 1.533 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	171	171	154	154	9.8	21.2			-59.2
2	164	164	202	202	2.8	26.8			-21.2
3	144	144	234	234	17.2	58.8			-17.2
4	158	158	170	170	3.2	5.2			-5.2
5	169	169	116	116	7.8	59.2			-3.2
6									2.8
7									7.8
8									9.8
9									26.8
10									58.8

Number normal was not significantly less in the test sediment WWD02-0-4-150205 than in the reference sediment (WWD-REF-150319) at $\alpha=0.10$. - 6 J1

Project Name: P865-4 Mytilus Number Normal

Sample: x1
 Samp ID: WWD02-4-8-150205
 Alias: NAS# 5245G
 Replicates: 5
 Mean: 166
 SD: 12.186
 Tr Mean: 166
 Trans SD: 12.186

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 175.2
 SD: 45.158
 Tr Mean: 175.2
 Trans SD: 45.158

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 21.461 SS: 8750.8 K: 5 b: 91.971 Alpha Level: 0.05 Calculated Value: 0.9666 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 8.8 Test Residual SD: 7.19 Ref. Residual Mean: 34.24 Ref. Residual SD: 23.953 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 2.2746 Critical Value: ≥ 1.860 Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 5 Experimental Alpha Level: 0.1 Calculated Value: 0.4398 Critical Value: ≥ 1.476 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	183	183	154	154	17	21.2			-59.2
2	160	160	202	202	6	26.8			-21.2
3	168	168	234	234	2	58.8			-16
4	150	150	170	170	16	5.2			-6
5	169	169	116	116	3	59.2			-5.2
6									2
7									3
8									17
9									26.8
10									58.8

Number normal was not significantly less in the test sediment WWD02-4-8-150205 than in the reference sediment (WWD-REF-150319) at $\alpha=0.10$. -631

Project Name: P865-4 Mytilus Number Normal

Sample: x1
 Samp ID: WWD05-0-4-150206
 Alias: NAS# 5231G
 Replicates: 5
 Mean: 151.4
 SD: 30.336
 Tr Mean: 151.4
 Trans SD: 30.336

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 175.2
 SD: 45.158
 Tr Mean: 175.2
 Trans SD: 45.158

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 24.961 SS: 11838 K: 5 b: 105.76 Alpha Level: 0.05 Calculated Value: 0.9448 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 21.36 Test Residual SD: 18.708 Ref. Residual Mean: 34.24 Ref. Residual SD: 23.953 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 0.9476 Critical Value: ≥ 1.860 Variances Homogeneous: Yes	Statistic: Student's t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x1 \geq x2$ Alternate: $x1 < x2$ Degrees of Freedom: 8 Experimental Alpha Level: 0.1 Calculated Value: 0.9783 Critical Value: ≥ 1.397 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	158	158	154	154	6.6	21.2			-59.2
2	171	171	202	202	19.6	26.8			-53.4
3	169	169	234	234	17.6	58.8			-21.2
4	98	98	170	170	53.4	5.2			-5.2
5	161	161	116	116	9.6	59.2			6.6
6									9.6
7									17.6
8									19.6
9									26.8
10									58.8

Number normal was not significantly less in the test sediment WWD05-0-4-150206 than in the reference sediment (WWD-REF-150319) at $\alpha=0.10$. -6J1

Project Name: P865-4 Mytilus Number Normal

Sample: x1
 Samp ID: WWD12-0-4-150206
 Alias: NAS# 5247G
 Replicates: 5
 Mean: 196
 SD: 12.062
 Tr Mean: 196
 Trans SD: 12.062

Ref Samp: x2
 Ref ID: WWD-REF-150319
 Alias: NAS# 5230G
 Replicates: 5
 Mean: 175.2
 SD: 45.158
 Tr Mean: 175.2
 Trans SD: 45.158

Shapiro-Wilk Results:	Levene's Results:	Test Results:
Residual Mean: 0 Residual SD: 21.446 SS: 8738.8 K: 5 b: 91.739 Alpha Level: 0.05 Calculated Value: 0.9631 Critical Value: ≤ 0.842 Normally Distributed: Yes Override Option: N/A	Test Residual Mean: 9.2 Test Residual SD: 6.301 Ref. Residual Mean: 34.24 Ref. Residual SD: 23.953 Deg. of Freedom: 8 Alpha Level: 0.1 Calculated Value: 2.2607 Critical Value: ≥ 1.860 Variances Homogeneous: No	Statistic: Approximate t Balanced Design: Yes Transformation: No Transformation Experimental Hypothesis Null: $x_1 \geq x_2$ Alternate: $x_1 < x_2$ Degrees of Freedom: 5 Experimental Alpha Level: 0.1 Calculated Value: -0.9951 Critical Value: ≥ 1.476 Accept Null Hypothesis: Yes Power: Min. Difference for Power:

Replicate Number	Test Data	Trans. Test Data	Reference Data	Trans. Reference Data	Levene's Test Residuals	Levene's Reference Residuals	Mann-Whitney Ranks	Rankits	Shapiro-Wilk Residuals
1	213	213	154	154	17	21.2			-59.2
2	181	181	202	202	15	26.8			-21.2
3	202	202	234	234	6	58.8			-15
4	192	192	170	170	4	5.2			-5.2
5	192	192	116	116	4	59.2			-4
6									-4
7									6
8									17
9									26.8
10									58.8

Number normal was not significantly less in the test sediment WWD12-0-4-150206 than in the reference sediment 9WWD-REF-150319) at $\alpha=0.10$.

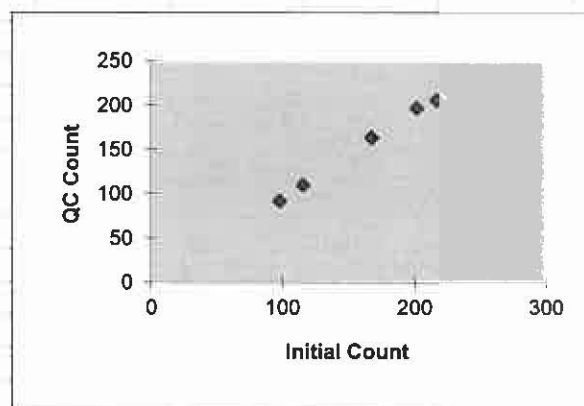
-631

Water Quality Data File														
NAS		CLIENT												
BKR	SMPL	DESCRIP	REP	DAY	TEMP	pH	SAL	DO	S	< or >	NH4+NH3	< or >	NH3	
10	swcontrol	swcontrol	6	0	15.0	8.0	28.0	8.1	<0.02	<	0.1	<	0.003	
14	5230G	WWD-REF-150319	6	0	15.1	8.0	28.0	7.7	<0.02		0.3		0.007	
17	5247G	WWD12-0-4-150206	6	0	15.0	8.0	29.0	7.7	<0.02	<	0.1	<	0.003	
23	5245G	WWD02-4-8-150205	6	0	15.1	8.1	28.0	7.5	<0.02		0.2		0.006	
28	5231G	WWD05-0-4-150206	6	0	15.3	8.1	29.0	7.3	<0.02		0.1		0.004	
34	5246G	WWD02-0-4-150205	6	0	15.3	8.1	29.0	7.3	<0.02		0.1		0.004	
10	swcontrol	swcontrol	6	1	15.1	8.1	28.0	7.9						
14	5230G	WWD-REF-150319	6	1	15.1	8.1	28.0	7.9						
17	5247G	WWD12-0-4-150206	6	1	15.0	8.1	28.5	7.9						
23	5245G	WWD02-4-8-150205	6	1	15.1	8.1	28.5	7.7						
28	5231G	WWD05-0-4-150206	6	1	15.3	8.1	28.0	7.9						
34	5246G	WWD02-0-4-150205	6	1	15.4	8.1	29.0	7.9						
10	swcontrol	swcontrol	6	2	15.1	8.2	28.0	8.0	<0.02	<	0.1	<	0.004	
14	5230G	WWD-REF-150319	6	2	15.1	8.1	28.0	8.0	<0.02		0.1		0.004	
17	5247G	WWD12-0-4-150206	6	2	15.1	8.1	28.0	8.0	<0.02	<	0.1	<	0.003	
23	5245G	WWD02-4-8-150205	6	2	15.2	8.1	29.0	8.0	<0.02		0.1		0.003	
28	5231G	WWD05-0-4-150206	6	2	15.4	8.1	28.5	8.1	<0.02	<	0.1	<	0.003	
34	5246G	WWD02-0-4-150205	6	2	15.4	8.1	28.0	8.0	<0.02		0.1		0.004	
					Mean	15.2	8.1	28.4	7.8	—	—		—	
					SD	0.1	0.0	0.4	0.2	—	—		—	
					n	18	18	18	18	12	12		12	
					Min	15.0	8.0	28.0	7.3	<0.02	<	0.1	<	0.003
					Max	15.4	8.2	29.0	8.1	<0.02		0.3		0.007

data entry verified against laboratory bench sheets 5-26-15 jrf

Comparison of Initial Counts and QC Counts

Beaker No.	Normal Larvae				cv(normal)
	Initial Count		QC Count		
	Normal	Abnormal	Normal	Abnormal	
7	202	2	197	4	2
15	98	18	92	13	4
19	217	28	206	25	4
22	116	3	110	8	4
30	168	3	164	4	2



Intercept	-3.05635
Slope	0.979128
r square	0.997654

AMMONIA EXPOSURE BENCHSHEETS AND ANALYSIS

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

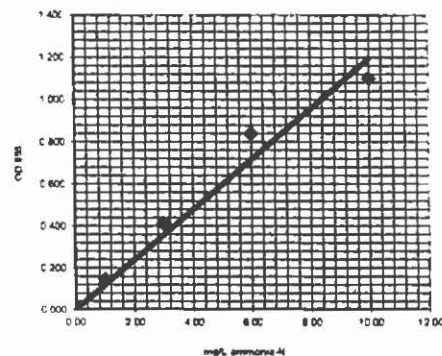
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449	3.71		
3.0 mg/L spike dupl.	----	0.444	3.67		
5.0 mg/L 2nd source		0.750	6.20		
1 5230G	5	0.189	7.81		
2 5231G	5	0.077	3.18		
3 5245G	5	0.283	11.70		
4 5246G	5	0.090	3.72		
5 5247G	5	0.025	1.03		

$$y = 0.1193x$$

$$R^2 = 0.9492$$

Standard Curve



Reporting limit (mg/L) = 0.50

Recovery (%) = 123.1

Precision (RPD) = 1.12

2nd source (%) = 124.0

Sample volume (ml) 0.10

Dilution factor 5

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)

☒ Bulk Sediment Porewaters☐ Test Beaker Porewaters☐ Overlying Water

Analyst:

RSC/JB

Date analysed:

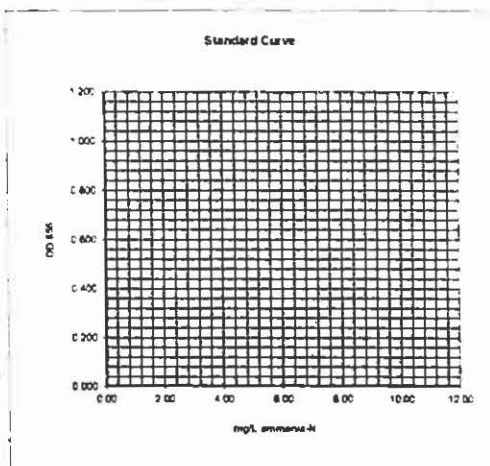
3/24/2015

Handwritten initials: RSC

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD655	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.145	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.413	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.840	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.100	10.00		
3.0 mg/L spike	----	0.449			
3.0 mg/L spike dupl.	----	0.444			
5.0 mg/L 2nd source		0.750			
1 5230G	1	0.189		7.5	27.0
2 5231G	1	0.077		7.8	29.0
3 5245G	1	0.283		8.1	29.5
4 5246G	1	0.090		8.0	29.5
5 5247G	1	0.025		8.0	29.0



Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: P865

Test Day:

Species:

Sample Type (check)☒ Bulk Sediment Porewaters☐ Test Beaker Porewaters☐ Overlying Water

Analyst:

Date analysed:

RSC/JB

3/24/2015

Handwritten initials: RSC
Handwritten initials: JB

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

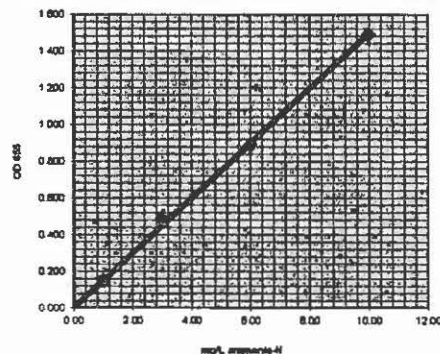
Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.150	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.485	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.890	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.490	10.00		
3.0 mg/L spike	----	0.495	3.31		
3.0 mg/L spike dupl.	----	0.475	3.17		
5.0 mg/L 2nd source		0.700	4.68		

$$y = 0.1496x$$

$$R^2 = 0.9986$$

Standard Curve



1	Day 0 (4-22-15)				
2	10	1	0.002	ND	
3	14	1	0.038	0.25	
4	17	1	0.010	ND	
5	23	1	0.026	0.17	
6	28	1	0.020	0.13	
7	34	1	0.018	0.12	
8					
9	Day 2 (4-24-15)				
10	10	1	0.009	ND	
11	14	1	0.017	0.11	
12	17	1	0.012	ND	
13	23	1	0.015	0.10	
14	28	1	0.010	ND	
15	34	1	0.018	0.12	

Reporting limit (mg/L) = 0.10

Recovery (%) = 108.0

Precision (RPD) = 4.12

2nd source (%) = 93.5

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-4

Test Day: 0 & 2

Species: *Mytilus***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst:

JB

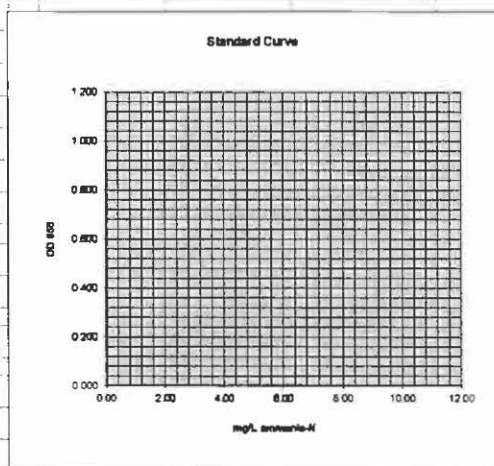
Date analyzed:

4/24/2015

Total Ammonia-N in Sediment Pore Water: Computation Worksheet **Salicylate Method (SOP #5492)**

Result

Sample description	Dilution factor	OD ₆₅₅	NH ₃ -N (mg/L)	pH	Salinity (ppt)
Blank	----	----	----		
1.0 mg/L NH ₃ -N Std.	----	0.150	1.00		
3.0 mg/L NH ₃ -N Std.	----	0.485	3.00		
6.0 mg/L NH ₃ -N Std.	----	0.890	6.00		
10.0 mg/L NH ₃ -N Std.	----	1.490	10.00		
3.0 mg/L spike	----	0.495			
3.0 mg/L spike dupl.	----	0.495			
5.0 mg/L 2nd source		0.700			



1	Day 0 (4-22-15)		
2	10	1	0.002
3	14	1	0.038
4	17	1	0.610
5	23	1	0.026
6	28	1	0.020
7	34	1	0.018
8			
9	Day 2 (4-24-15)		
10	10	1	0.009
11	14	1	0.017
12	17	1	0.012
13	23	1	0.015
14	28	1	0.010
15	34	1	0.018

Reporting limit (mg/L) = 0.10

Recovery (%) = #VALUE!

Precision (RPD) = #VALUE!

2nd source (%) = #VALUE!

Sample volume (ml): 0.50

Dilution factor 1

Sample Set Description:

Test No.: 865-4

Test Day: 0 & 2

Species: *Mytilus***Sample Type (check)**

Bulk Sediment Porewaters

Test Beaker Porewaters

X Overlying Water

Analyst:

JB

Date analyzed:

4/24/2015

CHAIN-OF-CUSTODY RECORDS

CUSTODY TRANSFER

Printed: 03/19/15

ARI Job No: ZV36

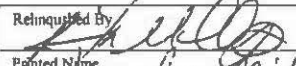

ANALYTICAL
RESOURCES
INCORPORATED4611 South 134th Place, Suite 100
Tukwila WA 98168
206-695-6200 206-695-6201 (fax)

ARI Project Manager: Cheronne Oreiro	Client Contact: Dan Berlin	Sampling Event: 110403-01.02	Samples Received: 02/05/15
	Client: Anchor QEA, LLC.	Project: West Waterway Sediment Char	Sample Site: NA

LOGNUM ARI ID	CLIENT ID	MATRIX	# CONTAINERS	ANALYTICAL REQUEST	ANALYTICAL REQUEST	ANALYTICAL REQUEST	COMMENTS
15-2108 ZV36D	WWD02-0-4-150205	Sediment	1	Bioassay			NAS # 52466
15-2109 ZV36E	WWD02-4-8-150205	Sediment	1	Bioassay			NAS # 52456

TEMP: 14.5°C

PAGE 20 OF 28

Comments/Special Instructions 128326950355612155 128326950356908763 128326950356171571 128326950356999988	Relinquished By  Printed Name Jennifer M. Hays Company ARI Date/Time 3/19/15 1400	Received by (Signature)  Printed Name GERALD IRISSARI Company NAS Date/Time 3-23-15 / 1325	Relinquished By Printed Name Company Date/Time 	Received by (Signature) Printed Name Company Date/Time
---	---	--	---	--

CUSTODY TRANSFER

Printed: 03/19/15

ARI Job No: ZV66



4611 South 134th Place, Suite 100
Tukwila WA 98168
206-695-6200 206-695-6201 (fax)

ARI Project Manager: Cheronne Oreiro	Client Contact: Dan Berlin	Sampling Event: 110103-01.02	Samples Received: 02/06/15
	Client: Anchor QEA, LLC.	Project: West Waterway Sediment Char	Sample Site: NA

LOGNUM ARI ID	CLIENT ID	MATRIX	# CONTAINERS	ANALYTICAL REQUEST	ANALYTICAL REQUEST	ANALYTICAL REQUEST	COMMENTS
15-2221 ZV66A	WWD05-0-4-150206	Sediment	1	Bioassay			NAS# 5231G
15-2224 ZV66D	WWD12-0-4-150206	Sediment	1	Bioassay			NAS# 5247G

TEMP: 14.0 °C

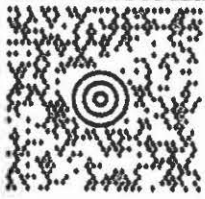
PAGE 21 OF 28

Comments/Special Instructions	Relinquished By <i>[Signature]</i>	Received by (Signature) <i>[Signature]</i>	Relinquished By	Received by (Signature)
	Printed Name Jennifer Millsap	Printed Name GERALD IRISSARRI	Printed Name	Printed Name
	Company ARI	Company NAS	Company	Company
	Date/Time 3/19/15 1400	Date/Time 3-23-15 1315	Date/Time	Date/Time

SHIP
TO:

20/Mar/2015 03:10 9729

**NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639**



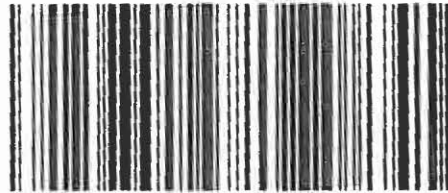
OR 973 2-01



128328850358808763

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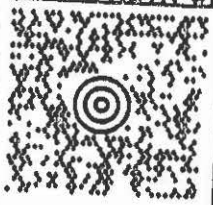


SHKSHHL LICENSE US 9729 MAR 20 03:09:12 2015 R1P 1.3.1 ZP4505

SHIP
TO:

20/Mar/2015 03:37 9729

NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639



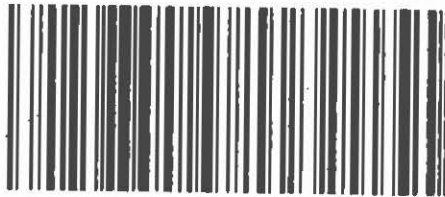
OR 973 2-01



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SNK5MHL

LICENSE

US 9729

MAR 20 03:36:19 2015

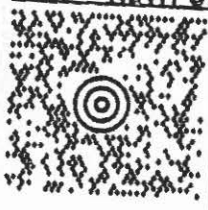
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2P4505

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TO:

20/Mar/2016 03:43 9729

NORTHWESTERN AQUATIC SCIENCES
SAMPLE RECEIVING
3814 YAQUINA BAY RD
NEWPORT OR 97365 9639



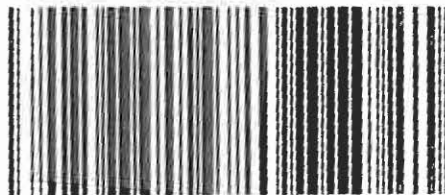
OR 973 2-01



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UPS GROUND

TRACKING #: 1Z 832 695 03 5699 9988



SHKSHL LICENSE US 9729 MAR 20 03:42:54 2016 HIP 14.3.1 2P4505

SAMPLE RECEIVING
(206) 891-6200
ARI LABS INC
1611 S 164TH PL
TUKWILA WA 98168-3212

36 DLS

(01)

SHIP TO:

SAMPLE RECEIVING

(541) 267-7225

WESTERN AQUATIC RESOURCES

10000 BOY RD

97366 9638

TEMP 15-5°C

MAS# 52316

373 2-01

CUSTODY SEAL

Date

3/14/15

Signature

[Signature]

Thermo
SCIENTIFIC

90006

CUSTODY SEAL

Date

3/19/15

Signature

[Handwritten Signature]

Thermo
SCIENTIFIC

90009

CUSTC

'SEA L

Date

3/1

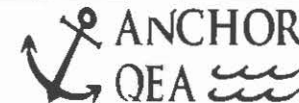
Signature

[Handwritten Signature]

10/9/15

Thermo
SCIENTIFIC

90009



Date: 3/19/15

Laboratory: Analytical Resources, Inc.

Project Name: West Waterway Sediment Characterization

Project Number: 110003-01.02

Project Manager: Dan Berlin

Phone Number: (206) 903-3322

Shipment Method: Delivery

Line	Field Sample ID	Collection Date/Time	Matrix	No. of Containers	Sediment and Field QC														Comments
					Total Metals	SVOCs/PAHs/PCBs/pesticides	Bulk TBT	Dioxin/furans	Grain Size	Ammonia	TS/TOC/TVS	Total Sulfides	Archive (Chemistry)	Asbestos (Bioassay)					
1	WWD-REF-150319	3/19/15 1105	SE	2										X					MAR# 52306
2			SE																
3			SE																
4			SE																
5			SE																
6			SE																
7			SE																
8			SE																TEMP BLANK = 4.20C
9			SE																
10			SE																
11			SE																
12			SE																
13			SE																
14			SE																

1 See project SAP/QAPP for analyte lists and test methods

2 email sample confirmation report to labdata@anchorqea.com

Additional notes/comments:

NITROGEN IN HEADSPACE AS PRESERVATIVE

Relinquished By:	Company: Anchor QEA LLC.	Received By:	Company: ARI
<u>SARA Bitterman</u>	<u>3/19/15 15:07</u>	<u>Guy Buhle</u>	<u>MAS 3-2075 1135</u>
Signature/Printed Name	Date/Time	Signature/Printed Name	Date/Time

Relinquished By:	Company:	Received By:	Company:
Signature/Printed Name	Date/Time	Signature/Printed Name	Date/Time

FedEx Express **US Airbill**

8656 3169 3053

0200

Form 10 No

FedEx Retrieval Copy

1 From
 Date 4/1/94 Sender's FedEx Account Number 2118 11224
 Sender's Name Amazon.com Phone 206 461 1000
 Company Amazon.com
 Address 410 9th Ave Dept./Floor/Suite/Room
 City Seattle State WA ZIP 98101

2 Your Internal Billing Reference www

3 To
 Recipient's Name David J. Smith Phone 415 461 1000
 Company Northwestern Pacific Science
 Recipient's Address 3514 Parkway Bay Dept./Floor/Suite/Room
 Address 3514 Parkway Bay
 City Newport State OR ZIP 97131



8656 3169 3053

4a Express Package Service
☒ **FedEx Priority Overnight** Next business morning* Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
☐ **FedEx Standard Overnight** Next business afternoon* Saturday Delivery NOT available.
☐ **FedEx 2Day** Second business day* Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected. FedEx Envelope rate not available. Minimum charge One pound rate.
☐ **FedEx Express Saver** Third business day* Saturday Delivery NOT available.
Packages up to 150 lbs.
☐ **FedEx First Overnight** Earliest next business morning delivery to select locations* Saturday Delivery NOT available.
4b Express Freight Service
☐ **FedEx 1Day Freight** Next business day** Prices shipments will be delivered on Monday unless SATURDAY Delivery is selected.
☐ **FedEx 2Day Freight** Second business day** Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
☐ **FedEx 3Day Freight** Third business day** Saturday Delivery NOT available.
Packages over 150 lbs.
 * Call for Confirmation. ** To meet locations.

5 Packaging
☐ **FedEx Envelope** ☐ **FedEx Pak*** Includes FedEx Small Pak, FedEx Large Pak, and FedEx Saver Pak. ☐ **FedEx Box** ☐ **FedEx Tube** ☒ **Other**
 * Declared value limit \$500.

6 Special Handling
☐ **SATURDAY Delivery** Not available for FedEx Standard Overnight, FedEx First Overnight, FedEx Express Saver, or FedEx 2Day Freight.
☐ **HOLD Weekday at FedEx Location** Not available for FedEx First Overnight.
☐ **HOLD Saturday at FedEx Location** Available ONLY for FedEx Priority, Overnight, and FedEx 2Day to select locations.
 Does this shipment contain dangerous goods? One box must be checked.
☒ **No** ☐ **Yes** As per attached Shipper's Declaration ☐ **Yes** Shipper's Declaration not required.
 Dangerous goods (including dry ice) cannot be shipped in FedEx packaging. ☐ **Dry Ice** Dry Ice & UN 1845 ☐ **Cargo Aircraft Only**

7 Payment Bill to: Enter FedEx Acct No. or Credit Card No. below.
☒ **Sender** Acct No. in Section 1 will be billed. ☐ **Recipient** ☐ **Third Party** ☐ **Credit Card** ☐ **Cash/Check** Obtain Recip Acct No.
 Total Packages 1 Total Weight 3.6
 *Our liability is limited to \$100 unless you declare a higher value. See the current FedEx Service Guide for details. Credit Card Act.

8 Residential Delivery Signature Options If you require a signature, check Direct or Indirect.
☐ **No Signature Required** Package may be left without obtaining a signature for delivery.
☐ **Direct Signature** Someone at recipient's address may sign for delivery. Fee applies.
☐ **Indirect Signature** If no one is available at recipient's address, someone at a neighboring address may sign for delivery. Fee applies.
520
 Rev. Date 10/93-Port #158211-©1994-2005 FedEx-PRINTED IN U.S.A. SRY

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

Test No. 999-3413 Client: QC Test

Investigator

REVIEWED
PAGES 1-9
-631

STUDY MANAGEMENT

Client: QC TestClient's Study Monitor: QC TestTesting Laboratory: Northwestern Aquatic SciencesTest Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Mgr./Study Dir. G.J. Irissarri ⁶³¹QA Officer L.K. Nemeth1. Yves Nakamura2. Lauren Brady ^{LB}3. 4. D.S. Caldwell ^{DS}

Study Schedule:

Test Beginning: 4-22-15 ¹³⁴⁰Test Ending: 4-24-15¹⁶²⁰ ⁶³¹
⁴⁻²⁴⁻¹⁵

TEST MATERIAL

Description: Copper as CuSO₄·5H₂O, Argent Lot# 0195,NAS Sample No. 1.0 mg/ml stock prepared: 6-19-14Date of Collection: Date of Receipt: Temperature (deg C): pH: Dissolved oxygen (mg/L): Conductivity (umhos/cm): Hardness (mg/L): Alkalinity (mg/L): Salinity (ppt): Total chlorine (mg/L): Total ammonia-N (mg/L):

DILUTION WATER

Description: Yaquina Bay, OR SeawaterDate of Collection: 4-21-15 Salinity (ppt) 30.0 pH 8.1Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q® deionized water

TEST ORGANISMS

Species: Mytilus galloprovincialisDate Received: 4-21-15Source: Taylor's SHELFISH FARMS, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
4-22-15	10.7	7.8	33.0	7.7	Held outside in trays of flowing seawater
Mean					
S.D.					
(N)					

Photoperiod during acclimation: Outdoor ambient conditions

Test No. 999-3413 Client _____ QC Test _____ Investigator _____**SPAWNING AND GAMETE HANDLING**

Spawning: Initial: 0915 Final: 1130 Fertilization: 1220
 Number of organisms used: females: 3 males: 3
 Egg Dilution (1 ml diluted to 100 ml):
 Count/ml of dilution: 1. 49 2. 45 3. 49 Mean: 47.7
 Dilution factor = DF (mean $\times \frac{100}{2500}$) = 0.95
 FURTHER DILUTED 10:1 w/ DILUTION WATER

TEST PROCEDURES AND CONDITIONSTest concentrations (50% series recommended): 64, 32, 16, 8, 4, 2, 1 and 0 ug/L

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4
 Organisms/ml (15-30): 22.9 Test water changes: None Aeration during test: None
 Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt
 Temperature: 20 +/- 1 °C, oysters; 16 +/- 1 °C, mussels Beaker placement: Stratified randomization

RANDOMIZATION CHART

A	7	64	12	Ø	32	1	8	2		
B	8	16	1	4	14	2	32	Ø		
C	32	4	2	1	8	Ø	64	16		
D	Ø	8	32	16	2	64	4	1		

PREPARATION OF TEST SOLUTIONS4-22-15
651

Test Conc. (Cu, ug/L)	ml of working stock #2 (2 ug/mL)	Dilution water (ml/100mL)
64	3.2	Brought up to a
32	1.6	final volume of
16	0.8	100 ml with
8	0.4	dilution water.
4	0.2	
2	0.1	
1	0.05	
0	0	

1st working stock made by 1:99 (1.0 mL \uparrow 100mL) dilution of concentrated 1 mg/mL stock solution. Final concentration 10 ug/mL.

2nd working stock made (working stock #2) made by 20:80 (20 mL \uparrow 100mL) dilution of 1st working stock. Final concentration 2 ug/mL.

Comments:

Test No. 999-3413 Client QC Test Investigator

WATER QUALITY DATADate: 4-22-15 initials: Date: 4-24-15 initials:

Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	15.1	8.0	29.5	8.1	15.3	8.1	29.5	8.0
32	15.1	8.0	29.5	8.1	15.3	8.1	30.0	8.0
16	15.1	8.0	30.0	8.1	15.2	8.1	30.5	8.0
8	15.1	8.0	30.0	8.1	15.3	8.1	30.5	8.0
4	15.0	8.0	30.0	8.1	15.3	8.1	30.5	8.0
2	15.1	8.0	30.0	8.1	15.3	8.1	30.5	8.0
1	15.1	8.0	30.0	8.1	15.3	8.1	30.5	8.0
Control	15.2	8.0	30.0	8.1	15.4	8.1	30.5	8.0
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:

Temperature (°C):

pH:

Salinity (ppt):

DO (mg/L):

Mean

SD

N

15.2

0.1

16

8.1

0.1

16

30.1

0.4

16

8.1

0.1

16

Room/ Water bath temperature: (°C)

Day 0:

Day 0:

Day 1:

Day 1:

Day 2:

Day 2:

LARVAL COUNT DATA

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	0	9	0	7	0	9	0	8
32	0	98	0	119	1	101	0	90
16	1	201	0	191	7	173	0	158
8	123	75	140	79	101	84	111	81
4	167	15	196	23	185	29	157	16
2	197	20	182	15	174	28	172	18
1	182	11	174	17	160	20	199	23
Control	203	20	183	15	183	13	186	16
Brine control	---	---	---	---	---	---	---	---
Zero time	220	227	234	231	223	240	---	---

Zero time: Mean 229 SD 7 N 6CV=(sd/mean)x100 3.2%

Remarks:



TAYLOR SHELLFISH FARMS

SE 130 LYNCH RD, SHELTON, WA 98584
PH: (360) 426-6178 FAX: (360) 426-3643
WASHINGTON CERTIFICATION #: WA 46SP



10.55
UNPROCESSED MUSSELS

Original Harvesters Cert#: WA 46 SP
Original Harvest Date: 4/19/2015
Final Harvest Date: 4/19/2015
Harvested In: Washington State
Harvest Area: Totten Inlet
Wet Stored:

Airbill #

SHIP TO:

NW AQUATIC

Cert #	
PO #	
ITEM #	

161

Rec'd 4-21-15

Perishable Keep Refrigerated 2-5 C°
Farm Raised Shellfish - Product of USA

Taylor Shellfish Farms

Shelton, WA 98584
360-426-6178

WET STORED:
UNPROCESSED MUSSELS

SHIP TO: NW AQUATIC
HARVEST DATE: 4/19/2015
HARVESTED IN: Washington State
HARVEST AREA: Totten Inlet
QUANTITY & TYPE: 10.55

CETIS Summary Report

Report Date: 28 Apr-15 14:02 (p 1 of 2)
 Test Code: 999-3413 00-3213-1743

Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Batch ID:	05-5894-3612	Test Type:	Development-Survival			Analyst:					
Start Date:	22 Apr-15 13:40	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Yaquina Bay Seawater				
Ending Date:	24 Apr-15 16:20	Species:	Mytilis galloprovincialis			Brine:					
Duration:	51h	Source:	Taylor Shellfish Farms, WA			Age:					
Sample ID:	17-4204-7293	Code:	67D5883D			Client:	Internal Lab				
Sample Date:	22 Apr-15 13:40	Material:	Copper sulfate			Project:					
Receive Date:	22 Apr-15 13:40	Source:	Reference Toxicant								
Sample Age:	NA	Station:									
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
08-5568-5521	Combined Proportion Norm	4	8	5.657	11.9%		Dunnett Multiple Comparison Test				
10-6761-5821	Proportion Normal	4	8	5.657	4.96%		Dunnett Multiple Comparison Test				
21-4700-9842	Proportion Survived	16	32	22.63	13.8%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
05-1313-3574	Combined Proportion Norm	EC25	6.413	5.015	7.719		Linear Interpolation (ICPIN)				
14-0335-9169	Combined Proportion Norm	EC50	8.585	8.348	8.829		Trimmed Spearman-Kärber				
01-3847-8460	Proportion Normal	EC25	6.743	6.179	7.351		Linear Interpolation (ICPIN)				
14-5203-5608	Proportion Normal	EC50	8.72	8.487	8.959		Trimmed Spearman-Kärber				
04-8658-6487	Proportion Survived	EC50	30.47	29.57	31.4		Trimmed Spearman-Kärber				
Combined Proportion Normal Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.8242	0.7575	0.891	0.7991	0.8865	0.02097	0.04194	5.09%	0.0%
1		4	0.7806	0.6675	0.8937	0.6987	0.869	0.03554	0.07108	9.11%	5.3%
2		4	0.7915	0.7126	0.8704	0.7511	0.8603	0.02479	0.04958	6.26%	3.97%
4		4	0.7697	0.6478	0.8915	0.6856	0.8559	0.03829	0.07659	9.95%	6.62%
8		4	0.5186	0.402	0.6352	0.441	0.6114	0.03664	0.07328	14.13%	37.09%
16		4	0.008734	0	0.03213	0	0.03057	0.00735	0.0147	168.3%	98.94%
32		4	0.001092	0	0.004566	0	0.004367	0.001092	0.002183	200.0%	99.87%
64		4	0	0	0	0	0	0	0		100.0%
Proportion Normal Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.9223	0.9069	0.9376	0.9103	0.9337	0.004822	0.009645	1.05%	0.0%
1		4	0.9098	0.8717	0.9479	0.8889	0.943	0.01198	0.02395	2.63%	1.35%
2		4	0.8996	0.857	0.9422	0.8614	0.9239	0.01338	0.02676	2.98%	2.46%
4		4	0.8961	0.8595	0.9328	0.8645	0.9176	0.01152	0.02304	2.57%	2.83%
8		4	0.5961	0.529	0.6632	0.5459	0.6393	0.02108	0.04216	7.07%	35.36%
16		4	0.01096	0	0.04082	0	0.03889	0.009383	0.01877	171.2%	98.81%
32		4	0.002451	0	0.01025	0	0.009804	0.002451	0.004902	200.0%	99.73%
64		4	0	0	0	0	0	0	0		100.0%
Proportion Survived Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.8941	0.8078	0.9804	0.8559	0.9738	0.02712	0.05423	6.07%	0.0%
1		4	0.8581	0.7335	0.9827	0.786	0.9694	0.03916	0.07832	9.13%	4.03%
2		4	0.8799	0.8004	0.9594	0.8297	0.9476	0.02499	0.04998	5.68%	1.59%
4		4	0.8603	0.7011	1	0.7555	0.9563	0.05001	0.1	11.63%	3.79%
8		4	0.8668	0.7649	0.9687	0.8079	0.9563	0.03202	0.06403	7.39%	3.05%
16		4	0.798	0.6675	0.9286	0.69	0.8821	0.04102	0.08204	10.28%	10.74%
32		4	0.4465	0.3615	0.5315	0.393	0.5197	0.0267	0.05341	11.96%	50.06%
64		4	0.03603	0.02937	0.04268	0.03057	0.0393	0.00209	0.004181	11.61%	95.97%

CETIS Summary Report

Report Date:

28 Apr-15 14:02 (p 2 of 2)

Test Code:

999-3413 00-3213-1743

Bivalve Larval Survival and Development Test					Northwestern Aquatic Sciences
Combined Proportion Normal Detail					
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.8865	0.7991	0.7991	0.8122
1		0.7948	0.7598	0.6987	0.869
2		0.8603	0.7948	0.7598	0.7511
4		0.7293	0.8559	0.8079	0.6856
8		0.5371	0.6114	0.441	0.4847
16		0.004367	0	0.03057	0
32		0	0	0.004367	0
64		0	0	0	0
Proportion Normal Detail					
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9103	0.9242	0.9337	0.9208
1		0.943	0.911	0.8889	0.8964
2		0.9078	0.9239	0.8614	0.9053
4		0.9176	0.895	0.8645	0.9075
8		0.6212	0.6393	0.5459	0.5781
16		0.00495	0	0.03889	0
32		0	0	0.009804	0
64		0	0	0	0
Proportion Survived Detail					
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9738	0.8646	0.8559	0.8821
1		0.8428	0.8341	0.786	0.9694
2		0.9476	0.8603	0.8821	0.8297
4		0.7948	0.9563	0.9345	0.7555
8		0.8646	0.9563	0.8079	0.8384
16		0.8821	0.8341	0.786	0.69
32		0.4279	0.5197	0.4454	0.393
64		0.0393	0.03057	0.0393	0.03493

CETIS Test Data Worksheet

Report Date: 28 Apr-15 13:59 (p 1 of 1)
 Test Code: 00-3213-1743/999-3413

Bivalve Larval Survival and Development Test								Northwestern Aquatic Sciences
Start Date: 22 Apr-15 13:40		Species: Mytilus galloprovincialis		Sample Code: 67D5883D				
End Date: 24 Apr-15 16:20		Protocol: EPA/600/R-95/136 (1995)		Sample Source: Reference Toxicant				
Sample Date: 22 Apr-15 13:40		Material: Copper sulfate		Sample Station:				
C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	30	229	223	223	203	
0	D	2	24	229	198	198	183	
0	D	3	3	229	196	196	183	
0	D	4	21	229	202	202	186	
1		1	23	229	193	193	182	
1		2	19	229	191	191	174	
1		3	32	229	180	180	160	
1		4	5	229	222	222	199	
2		1	4	229	217	217	197	
2		2	20	229	197	197	182	
2		3	22	229	202	202	174	
2		4	9	229	190	190	172	
4		1	27	229	182	182	167	
4		2	29	229	219	219	196	
4		3	1	229	214	214	185	
4		4	12	229	173	173	157	
8		1	31	229	198	198	123	
8		2	26	229	218	219	140	
8		3	13	229	185	185	101	
8		4	11	229	192	192	111	
16		1	8	229	202	202	1	
16		2	10	229	191	191	0	
16		3	16	229	180	180	7	
16		4	28	229	158	158	0	
32		1	2	229	98	98	0	
32		2	7	229	119	119	0	
32		3	17	229	102	102	1	
32		4	6	229	90	90	0	
64		1	25	229	9	9	0	
64		2	18	229	7	7	0	
64		3	15	229	9	9	0	
64		4	14	229	8	8	0	

data entry verified against laboratory bench sheets 5-26-15 jmk

Bivalve Larval Survival and Development Test

Northwestern Aquatic Sciences

Test Type: Development-Survival

Organism: *Mytilus galloprovincialis* (Bay Mussel)

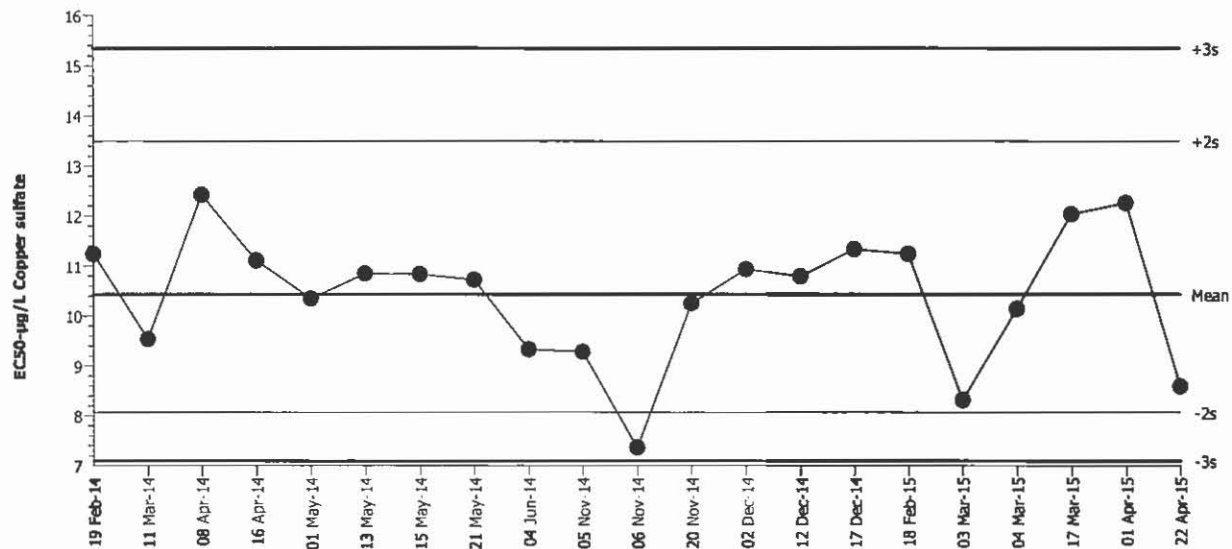
Material: Copper sulfate

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Combined Proportion Normal

Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 10.43

Count: 20

-2s Warning Limit: 8.067

-3s Action Limit: 7.094

Sigma: NA

CV: 13.70%

+2s Warning Limit: 13.49

+3s Action Limit: 15.35

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Feb	19	14:20	11.24	0.8077	0.5799			15-6679-6214	02-0497-8826
2		Mar	11	14:20	9.535	-0.8983	-0.7001			10-5884-3247	16-9220-6024
3		Apr	8	14:15	12.42	1.983	1.353			05-7663-6917	08-0903-6269
4			16	12:30	11.11	0.6718	0.4852			17-6418-7180	09-1930-9651
5		May	1	14:00	10.35	-0.08797	-0.06584			08-0421-9256	16-2250-1885
6			13	14:20	10.85	0.4152	0.3035			04-6559-4264	07-6758-4246
7			15	14:30	10.83	0.4002	0.2927			13-1994-5314	03-1132-1936
8			21	14:25	10.72	0.2853	0.2098			11-0393-3448	18-4806-9678
9		Jun	4	14:10	9.327	-1.106	-0.8717			01-1116-7690	18-6378-4512
10		Nov	5	15:40	9.28	-1.153	-0.9108			06-9286-9698	10-2134-9748
11			6	11:50	7.361	-3.073	-2.713	(-)		20-0794-9572	02-0939-9199
12			20	11:30	10.25	-0.1882	-0.1416			01-0435-6732	19-9107-3147
13		Dec	2	13:55	10.94	0.5021	0.3655			19-7704-4994	16-3002-0245
14			12	14:25	10.79	0.3567	0.2614			00-0454-5791	01-5367-4523
15			17	14:35	11.33	0.9012	0.6443			18-2606-6472	11-6810-3849
16	2015	Feb	18	14:50	11.24	0.8047	0.5777			09-3297-1712	13-0562-8762
17		Mar	3	13:25	8.311	-2.123	-1.769			04-6089-3353	10-0884-4050
18			4	14:10	10.14	-0.296	-0.2238			11-1749-6172	02-6924-0562
19			17	14:00	12.03	1.597	1.108			18-5112-6398	01-9547-9299
20		Apr	1	16:30	12.26	1.823	1.253			13-0618-2511	09-3363-8398
21			22	13:40	8.585	-1.848	-1.516			00-3213-1743	14-0335-9169

4-28-15
-62L